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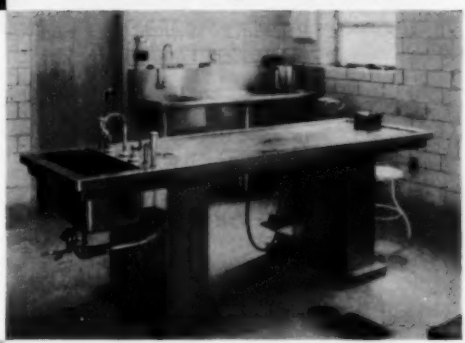

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Pulmonary Arteriosclerosis and Thromboembolism in Chronic Pulmonary Emphysema

JULES A. KERNEN, M.D., Indianapolis; ROBERT M. O'NEAL, M.D., and DAVID L. EDWARDS, M.D., St. Louis

Many patients with chronic pulmonary emphysema develop pulmonary hypertension and exhibit the clinical and pathological features of chronic cor pulmonale. In addition, many of these patients are found to have extensive arteriosclerosis of the small pulmonary arteries. The mutual interrelations of pulmonary hypertension and pulmonary arteriosclerosis in chronic pulmonary emphysema, and also in other diseases, have been emphasized by many authors, and each condition has been postulated as the cause of the other.[1-4] It has been asserted that the pulmonary hypertension is an important cause of the pulmonary arteriosclerosis. However, in recent years evidence has been presented indicating that advanced degrees of pulmonary arteriosclerosis are not necessarily associated with pulmonary hypertension and that thromboembolic processes may be a much more significant factor than pulmonary hypertension in the development of pulmonary arteriosclerosis. These reports have included morphologic studies of patients with congenital heart disease [5] and mitral stenosis [6] and experimental studies on laboratory animals subjected to pulmonary embolization by various techniques.[7-10] However, apparently no systematic morphologic study has been made of the possible interrelations of hypertension, arteriosclerosis, and thromboembolism in pulmonary arterial trees of patients with chronic pulmonary emphysema.

This report is an analysis of pulmonary arteriosclerosis, right ventricular hypertrophy, and pulmonary thromboembolism in 125 patients, autopsied either at Washington University or at Indiana University, who had died with diffuse pulmonary emphysema of moderate or advanced degree (either as a primary cause of death or as an incidental accessory finding at autopsy). As in previous studies,[5,6] we have used right ventricular hypertrophy as presumptive evidence that pulmonary hypertension had been present during life.

Materials and Methods

The complete autopsy files of Washington University School of Medicine and of the Indiana University Medical Center were examined; these represent a combined series of over 25,000 consecutive autopsies, performed during a period of more than 40 years. All cases in which a diagnosis of chronic pulmonary emphysema had been made were selected for initial screening. In both institutions, the degree of the emphysema in each case had been estimated as being "slight," "moderate," or "advanced." Only those cases that had been diagnosed as being of moderate or advanced degree were studied further. All cases which had some other chronic pulmonary disease, such as tuberculosis, bronchogenic carcinoma, or bronchiectasis, or which had significant valvular or

Submitted for publication Nov. 15, 1957.

From the Department of Pathology, Indiana University Medical Center (Dr. Kernen) and the Department of Pathology, Washington University School of Medicine (Drs. O'Neal and Edwards). During the preparation of this paper, Dr. Kernen was a trainee of the National Cancer Institute.

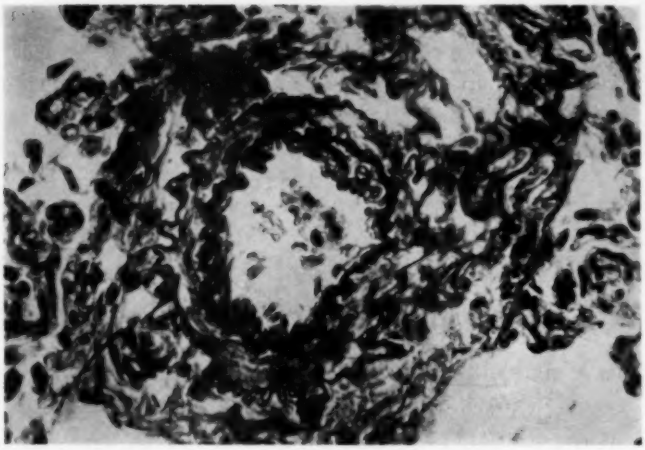

Fig. 1.—Grade 1 pulmonary arteriosclerosis in a small muscular artery. An eccentric fibrous intimal lesion, not as thick as the media, lies directly on the internal elastic lamella; × 375.

congenital cardiac disease were excluded. After the above screening, 125 cases of chronic pulmonary emphysema proved suitable for inclusion in the study.

From all of the selected cases, paraffin blocks of tissue of lung fixed in Zenker-formalin or neutral formalin were recut and stained, either with aldehyde-fuchsin-Van Gieson or with Verhoeff-Van Gieson stains.[11]

The microsections were examined, and the pertinent observations recorded, without reference to the protocols. Among the microscopic observations tabulated were extent and severity of arteriosclerosis in small muscular pulmonary arteries and presence of thromboembolic phenomena. Small muscular arteries contain a well-defined muscular media bordered by internal and external elastic lamellae. We have used the excellent classification of Brenner[12] and have preferred not to attempt a study of arterioles, since in the lung they do not have a continuous muscular coat and therefore cannot always be distinguished from venules.

In all cases, microscopic examination confirmed the gross diagnosis of pulmonary emphysema. They exhibited the three microscopic criteria suggested by Hartroft[13]: decrease in average alveolar depth, increase in average alveolar diameter, and flattening of the interalveolar septa.

The severity and frequency of arteriosclerosis in small muscular pulmonary arteries were graded separately, with use of a system previously described by one of us.[5] Severity of arteriosclerotic changes, chiefly fibrous intimal thickening, was graded as follows: Grade 1 indicates a lesion significantly less thick than the media of the same vessel (Fig. 1); Grade 2 indicates a lesion approximately as thick as the media (Fig. 2), and Grade 3 indicates a lesion definitely thicker than

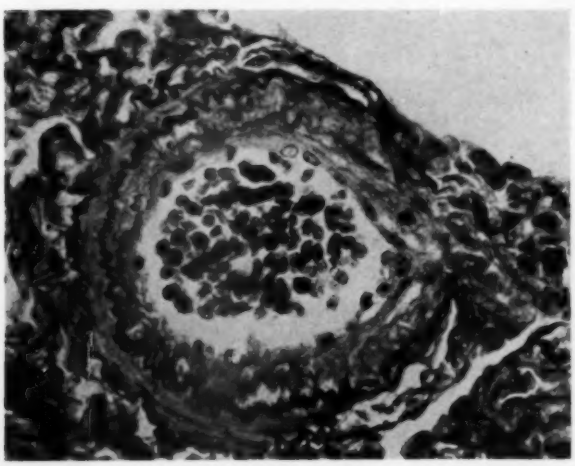

Fig. 2.—Grade 2 pulmonary arteriosclerosis in a small muscular artery. A concentric fibrous intimal lesion, approximately as thick as the media, narrows the lumen. Note the well-defined muscular media and wrinkled internal and external elastic lamellae; × 375.

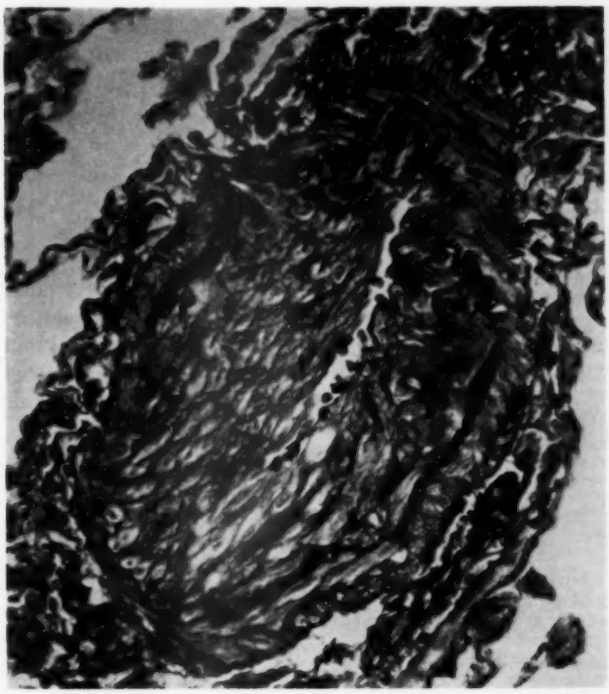

Fig. 3.—Grade 3 pulmonary arteriosclerosis in a small muscular artery. The fibrous intimal lesion is much thicker than the media, and the lumen is reduced to a narrow slit; × 300.

the media (Fig. 3). Frequency was graded in an analogous manner: Grade 1 for frequency indicates that one to three lesions were encountered in each tissue section; similarly, Grade 2 indicates four to six lesions, and Grade 3, more than six lesions in each tissue section. The average sizes of the tissue sections in each case were approximately the same, and two or three sections were studied in most cases. To facilitate statistical analyses, grades for frequency and severity were combined to obtain a single figure. If both frequency and severity were graded as 1, or if frequency was graded 1 and severity, 2, or vice versa, a combined grade of 1 ("slight") was assigned for combined frequency and severity. If both frequency and severity were graded 2, or if either was graded 3 and the other, 1, a combined grade of 2 ("moderate") was assigned. If both were graded as 3, or if either was graded as 3 and the other, graded as 2, a combined grade of 3 ("advanced") was assigned.

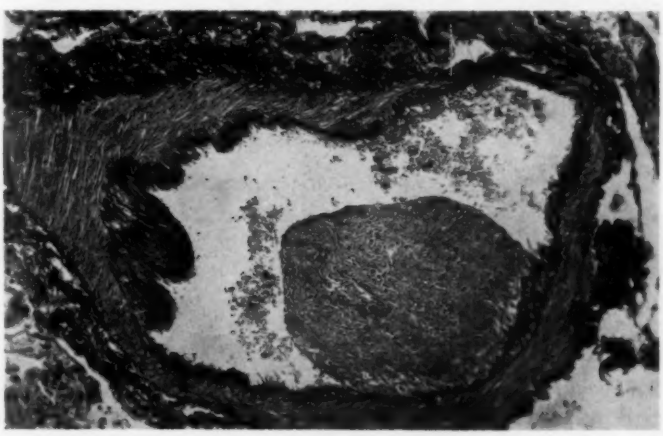

Fig. 4.—A clearly recognizable partially organized thrombus in a large muscular pulmonary artery. Reduced 28% from mag × 125.

From the autopsy protocols the following information was obtained: age, sex, measured thickness (in millimeters) of the wall of the right ventricle, degree of emphysema, and gross appearance of the lungs and pulmonary vessels. Pulmonary infarcts or clearly defined recent or organized thrombi (Fig. 4) in pulmonary arteries or arterioles, observed grossly or microscopically, were accepted as manifestations of pulmonary thromboembolic phenomena.

For comparison, a control group of 54 patients with neither significant cardiac nor chronic pulmonary disease was selected from recently performed autopsies. No attempt was made to select controls representatively in the entire 40 years of autopsies. But this control group was selected so that its age distribution would be the same as that of the emphysema group. Microsections from these cases were prepared and studied, and autopsy protocols abstracted exactly as for the emphysema cases.

In all statistical analyses, P values were calculated, with use of the χ^2-technique.

TABLE 1.—*Incidence of Right Ventricular Hypertrophy in Patients with Chronic Pulmonary Emphysema and in Controls*

Groups	Thickness of Right Ventricle			
	5 mm. & Less (Normal)		6 mm. & Over (Hypertrophy)	
	No.	%	No.	%
Controls (54 patients)	54	100	0	0
Patients with emphysema (125 patients)	80	64	45	36

Patients from both the control group and from the group with moderate or advanced pulmonary emphysema were divided into two classes, depending upon the thickness of their right ventricular myocardium. Measurements of 5 mm. or less were grouped together and were considered within normal limits. Those cases in which the right ventricular wall was greater than 5 mm. in thickness were also grouped together, and these were considered to represent cases of right ventricular hypertrophy.

Results

The right ventricles of 36% of patients with moderate or advanced chronic pulmonary emphysema were more than 5 mm. thick and hence were considered indicative of right ventricular hypertrophy (Table 1). Their thicknesses varied from 6 to 17 mm., with an average of 7.6 mm. None of the right ventricles in the control group measured more than 5 mm.

TABLE 2.—*Incidence of Thromboembolic Phenomena in Lungs in Patients with Chronic Pulmonary Emphysema and in Controls*

Groups	Thromboembolism in Lungs			
	Present		Absent	
	No.	%	No.	%
Controls (54 patients)	8	15	46	85
Patients with emphysema (125 patients)	28	22	97	78

Gross or microscopic evidence of pulmonary thromboembolization was found in 22% of patients with emphysema and in 15% of the control group (Table 2). This difference is not statistically significant ($P>0.2$).

Advanced pulmonary arteriosclerosis was present in 50% of patients with emphysema and in 33% of the patients in the control group (Table 3). This difference was not great enough to be of any genuine statistical significance ($P>0.05$).

Patients with pulmonary emphysema were divided into two groups, depending upon whether their right ventricles were greater than 5 mm. thick or were 5 mm. or less in thickness. The incidence of slight, moderate, or advanced arteriosclerosis was calculated for each group (Table 4). Forty-nine per cent of the group with right ventricular hypertrophy (right ventricles more than 5 mm. thick) had advanced pulmonary arteriosclerosis; the corresponding figure for the group of emphysema patients without right ventricular hypertrophy is 50%. The difference is obviously not significant.

The incidence of pulmonary thromboemboli was calculated in the two groups of patients with pulmonary emphysema (those with and without right ventricular hyper-

TABLE 3.—*Incidence of Arteriosclerosis in Small Muscular Pulmonary Arteries in Patients with Chronic Pulmonary Emphysema and in Controls*

Groups	Pulmonary Arteriosclerosis					
	Slight		Moderate		Advanced	
	No.	%	No.	%	No.	%
Controls (54 patients)	15	28	21	39	18	33
Patients with pulmonary emphysema (125 patients)	20	16	43	34	62	50

TABLE 4.—*Correlation of Thickness of Right Ventricle with Arteriosclerosis in Small Muscular Pulmonary Arteries in 125 Patients with Chronic Pulmonary Emphysema*

Thickness of Right Ventricle	Pulmonary Arteriosclerosis					
	Slight		Moderate		Advanced	
	No.	%	No.	%	No.	%
6 mm. and over (hypertrophy)	6	13	17	38	22	49
5 mm. and less (normal)	14	18	26	32	40	50

trophy; Table 5). Thromboemboli or infarcts were found in 24% of emphysema patients with right ventricular hypertrophy and in 21% of those without right ventricular hypertrophy. Again, this difference is not statistically significant.

Advanced pulmonary arteriosclerosis was found in 78% of emphysema patients with thromboemboli or infarcts but only in 42% of similar patients without evidence of thromboemboli or infarcts (Table 6). This difference is significant statistically ($P<0.01$).

TABLE 5.—*Correlation of Thickness of Right Ventricle with Thromboembolic Phenomena in Lungs in 125 Patients with Chronic Pulmonary Emphysema*

Thickness of Right Ventricle	Thromboembolism in Lungs			
	Present		Absent	
	No.	%	No.	%
6 mm. and over (hypertrophy)	11	24	34	76
5 mm. and less (normal)	17	21	63	79

Comment

The high incidence of right ventricular hypertrophy in patients with chronic pulmonary emphysema has again been emphasized in the comparison of our control series and emphysema series (Table 1). These data correlate well with direct measurements of pulmonary arterial pressure in patients with chronic pulmonary emphysema.[14,15] Cardiac catheterizations have revealed a high incidence of pulmonary hypertension in emphysema patients, especially in those with the complication of congestive heart failure.

Many authors have emphasized the high incidence of pulmonary arteriosclerosis associated with chronic pulmonary emphysema. They have invoked pulmonary hypertension as an important factor in the pathogenesis of the arteriosclerosis.[1-4] Our data (Table 3) indicate that arteriosclerosis of the small muscular pulmonary arteries is not significantly more frequent in patients with chronic pulmonary emphysema than in a control series of the same age distribution. It is reasonable to assume that in the control series, which included no cases of right ventricular hypertrophy, significant degrees of pulmonary hypertension had not been present. Pulmonary hypertension cannot be responsible for pulmonary arteriosclerosis, because the incidence of the latter is approximately the same whether or not the pulmonary hypertension is present. This conclusion is further strengthened by the finding (Table 4) that, within the group of patients with chronic pulmonary emphysema, pulmonary arteriosclerosis is found with approximately the same frequency in cases with and without right ventricular hypertrophy (and, hence, presumably with and without pulmonary hypertension).

As demonstrated in Table 6, our studies do indicate that there is a significant correlation between advanced pulmonary arteriosclerosis and the presence of pulmonary thromboemboli (78% of emphysema cases with pulmonary thromboembolization have advanced pulmonary arteriosclerosis, as compared with 42% of cases without pulmonary thromboembolization). If cases of both moderate and advanced pulmonary arteriosclerosis are considered together, the corresponding figures do not differ significantly (89% of cases with pulmonary thromboembolization, as compared with 83% of those

TABLE 6.—*Correlation of Thromboembolic Phenomena in Lungs with Arteriosclerosis in Small Muscular Pulmonary Arteries in 125 Patients with Chronic Pulmonary Emphysema*

Thromboemboli in Lungs	Pulmonary Arteriosclerosis					
	Slight		Moderate		Advanced	
	No.	%	No.	%	No.	%
Present	3	11	3	11	22	78
Absent	17	17	40	41	40	42

without). However, both in view of the small number of cases of slight and moderate (three each) pulmonary arteriosclerosis with pulmonary thromboembolization and emphysema and in view of the high incidence (39%) of moderate arteriosclerosis in the control group without emphysema, it seems both reasonable and imperative to consider only advanced pulmonary arteriosclerosis when seeking a correlation with pulmonary thromboembolization.

These studies offer further support for the hypothesis that thromboembolic phenomena are an important factor in the pathogenesis of pulmonary arteriosclerosis. Other evidence, both morphologic and experimental, has been offered to support this hypothesis. A high association (approximately 70%) of pulmonary arterial thrombi and arteriosclerotic lesions is found in autopsied cases of congenital heart disease, regardless of the type of anomaly present; many lesions are found that suggest the stages in the transformation of a fresh thrombus into an arteriosclerotic lesion.[5] Thomas et al.,[6] studying a series of 86 autopsied patients with mitral stenosis, found a highly significant correlation between the presence of arteriosclerosis of the small pulmonary arteries and the presence of thromboembolic phenomena in the lungs. Several investigators [7,8] have produced arteriosclerotic lesions experimentally in small pulmonary arteries of rabbits by repeated intravenous injections of blood clots. These rabbits developed pulmonary hypertension and right ventricular hypertrophy. A marked degree of pulmonary vascular obstruction and right ventricular hypertrophy has been obtained experimentally in rabbits by repeated intravenous injections of nonthrombotic obstructive bodies (small spherical plastic beads), but pulmonary arteriosclerotic lesions were not produced,[9] indicating that thromboembolic induced pulmonary arteriosclerotic lesions probably result from direct organization of thrombi or emboli and are not just secondary to pulmonary hypertension. Duguid [16] has long applied to the systemic circulation, also, the concept that arteriosclerotic plaques are produced by organization of thrombi.

The exact etiology of pulmonary hypertension in pulmonary emphysema remains undetermined. Various suggested etiologic factors can be grouped into two main categories: (1) organic structural relatively nonreversible changes which are morphologically demonstrable and (2) physiologic or functional relatively rapidly reversible factors. Among the former group are included anatomic reduction in the total cross-sectional area of the pulmonary capillary bed, secondary to changes in the alveolar septa, and increase in pulmonary vascular resistance caused by the narrowing of the pulmonary vascular tree by arteriosclerotic plaques and pulmonary thromboemboli.

The findings in this study indicate that neither pulmonary arteriosclerosis (Table 4) nor pulmonary thromboembolization (Table 5) can be an important factor in the pathogenesis of the pulmonary hypertension with chronic pulmonary emphysema, since the incidences of both arteriosclerosis and thromboembolization are approximately the same in emphysema cases with and without right ventricular hypertrophy (and hence, presumably, with and without pulmonary hypertension). This conclusion is further supported by the evidence that the incidence of pulmonary embolization (Table 2) and pulmonary arteriosclerosis (Table 3) is approximately the same in the control group (with no cases of right ventricular hypertrophy) and in the emphysema group. However, other studies, unrelated to pulmonary emphysema, have shown that occasional cases of pulmonary hypertension and chronic cor pulmonale when studied pathologically show only the occurrence of widespread organized thromboemboli in the pulmonary vessels,[4,17] indicating that long-standing pulmonary hypertension may in some cases result from repeated pulmonary embolization, although this is uncommon.

Several authors [15,18,19] have emphasized the role of physiologic hemodynamic factors

in the pathogenesis of pulmonary hypertension in patients with chronic pulmonary emphysema. Mounsey et al.[15] used the technique of cardiac catheterization to study 24 patients with severe chronic pulmonary emphysema. In those without clinical evidence of congestive heart failure, the systolic pressure in the right ventricle was found to be normal or only moderately increased, whereas in those cases with congestive heart failure, it was markedly increased in every case. As these patients recovered with therapy from the congestive failure, the right ventricular pressures returned to normal. Since the pulmonary hypertension in emphysema was reversible, they felt it could not be due solely to fixed anatomical changes in the vascular bed of the lungs. This agrees completely with the results of our study.

Anoxia has been suggested as an important factor in the pathogenesis of pulmonary hypertension in chronic pulmonary emphysema,[15,19] and Motley [20] demonstrated by cardiac catheterization the rapid development of pulmonary hypertension in normal persons breathing low oxygen (10%) mixtures for short periods of time. Rodbard [21] feels that bronchomotor hypertonus may be the primary factor in the pathogenesis of chronic pulmonary emphysema and the accompanying pulmonary hypertension. Finally, Liebow [22,23] has demonstrated by injection studies, in many chronic pulmonary diseases, the presence of precapillary anastomoses between the pulmonary and bronchial arterial circulations. In emphysema, he especially emphasizes the bronchopulmonary venous collateral circulation. The pulmonary arterial hypertension in chronic pulmonary emphysema may result, in part at least, from the shunting of arterial blood at systemic pressure from the bronchial arterial circulation into the pulmonary arterial circulation.

Summary

This study is an analysis of the mutual interrelations of right ventricular hypertrophy, arteriosclerosis of small muscular pulmonary arteries, and pulmonary thromboembolization in 125 cases of moderate or advanced chronic pulmonary emphysema. Right ventricular hypertrophy was considered to be presumptive evidence that pulmonary hypertension had been present. The controls were the lungs of 54 patients without significant chronic pulmonary or cardiac disease; these were selected in the same age distribution as the emphysema cases.

Approximately 36% of the emphysema patients had right ventricular hypertrophy, while this was found in none of the control group. The incidences of pulmonary thromboembolism and pulmonary arteriosclerosis did not differ significantly between the control group and the emphysema group.

In the emphysema group, there was no significant correlation either between pulmonary arteriosclerosis and right ventricular hypertrophy or between pulmonary thromboembolization and right ventricular hypertrophy. These findings suggest that pulmonary hypertension is not an important factor in the etiology of arteriosclerosis of small muscular pulmonary arteries in patients with chronic pulmonary emphysema. They also suggest that in pulmonary emphysema the hypertension is caused neither by the arteriosclerosis nor by the thromboemboli. Other possible causes of the hypertension are reviewed briefly.

There was significant correlation between the presence of advanced arteriosclerosis of the small muscular pulmonary arteries and the presence of thromboembolic phenomena. The results support the concept that thromboembolization is an important factor in the pathogenesis of pulmonary arteriosclerosis.

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REFERENCES

1. Friedberg, C. K.: Diseases of the Heart. Ed. 2, Philadelphia, W. B. Saunders Company, 1956.

2. Moschcowitz, E.: Hypertension of the Pulmonary Circulation: Its Causes, Dynamics, and Relation to Other Circulatory States, Am. J. M. Sc. 174:388 (Sept.) 1927.

3. Parker, R. L.: Pulmonary Emphysema: A Study of Its Relation to the Heart and Pulmonary Arterial System, Ann. Int. Med. 14:795 (Nov.) 1940.

4. Kuida, H.; Dammin, G.; Haynes, F.; Rappaport, E., and Dexter, L.: Primary Pulmonary Hypertension, Am. J. Med. 23:166 (Aug.) 1957.

5. O'Neal, R. M., and Thomas, W. A.: The Role of Pulmonary Hypertension and Thromboembolism in the Production of Pulmonary Arteriosclerosis, Circulation 12:370 (Sept.) 1955.

6. Thomas, W. A.; Lee, K. T.; Rabin, E. R., and O'Neal, R. M.: Mitral Stenosis and Pulmonary Arteriosclerosis: Correlation of Pulmonary Arteriosclerosis, Right Ventricular Hypertrophy, and Thromboembolism in Autopsied Patients Who Died with Mitral Stenosis, A. M. A. Arch. Path. 62:257 (Oct.) 1956.

7. Harrison, C. V.: Experimental Pulmonary Arteriosclerosis, Path. & Bact. 60:289 (April) 1948.

8. Barnard, P. J.: Pulmonary Arteriosclerosis and Cor Pulmonale Due to Recurrent Thromboembolism, Circulation 10:343 (Sept.) 1954.

9. Thomas, W. A.; O'Neal, R. M., and Lee, K. T.: Experimental Pulmonary Hypertension and Arteriosclerosis: Absence of Intimal Reaction in Pulmonary Arteries of Rabbits with Right Ventricular Hypertrophy Following Pulmonary Vascular Obstruction by Nonthrombotic Material (Plastic Beads), A. M. A. Arch. Path. 62:56 (Oct.) 1956.

10. Thomas, W. A.; O'Neal, R. M., and Lee, K. T.: Thromboembolism, Pulmonary Arteriosclerosis, and Fatty Meals: An Experimental Study of the Effect of Intermittent Fatty Meals on Thromboembolic-Induced Pulmonary Arteriosclerosis in Rabbits, A. M. A. Arch. Path. 61:380 (May) 1956.

11. Lillie, R. D.: Histopathologic Technic and Practical Histochemistry, Ed. 2, New York, The Blakiston Company, 1954.

12. Brenner, O.: Pathology of the Vessels of the Pulmonary Circulation: Part II, Arch. Int. Med. 56:457 (Sept.) 1935.

13. Hartroft, W. S.: The Microscopic Diagnosis of Pulmonary Emphysema, Am. J. Path. 21:889 (Sept.) 1945.

14. Yu, P. N. G.; Lovejoy, F., Jr.; Joos, H.; Nye, R., Jr., and McCann, W.: Studies on Pulmonary Hypertension: I. Pulmonary Circulatory Dynamics in Patients with Pulmonary Emphysema at Rest, J. Clin. Invest. 32:130 (Feb.) 1953.

15. Mounsey, J. P. D.; Ritzmann, L.; Selverstone, N.; Briscoe, W., and McLeMore, G. A.: Circulatory Changes in Severe Pulmonary Emphysema, Brit. Heart J. 14:153 (April) 1952.

16. Duguid, J. B.: The Etiology of Atherosclerosis, Practitioner 175:241 (Sept.) 1955.

17. Owen, W. R.; Thomas, W. A.; Castleman, B., and Bland E.: Unrecognized Emboli to the Lungs with Subsequent Cor Pulmonale, New England J. Med. 249:919 (Dec. 3) 1953.

18. Ferrer, M. I., and Harvey, R. M.: The Etiology of Secondary Pulmonary Hypertension, Bull. New York Acad. Med. 30:208 (March) 1954.

19. Mounsey, J. P. D.: Emphysema Heart Disease, Brit. J. Tuberc. 48:63 (Jan.) 1954.

20. Motley, H.; Cournand, A.; Werko, L.; Himmelstein, A., and Dresdale, D.: The Influence of Short Periods of Induced Acute Anoxia upon Pulmonary Artery Pressures in Man, Am. J. Physiol. 150:315 (Aug.) 1947.

21. Rodbard, S.: Bronchomotor Tone: A Neglected Factor in the Regulation of the Pulmonary Circulation, Am. J. Med. 15:356 (Sept.) 1953.

23. Liebow, A. A.: The Bronchopulmonary Venous Collateral Circulation with Special Reference to Emphysema, Am. J. Path. 29:251 (March-April) 1953.

23. Liebow, A.: Some Aspects of Collateral Circulation of the Lung, Bull. New York Acad. Med 30:66 (Jan.) 1954.

Histochemical Studies of Necrosis of Mouse Liver in Vitro

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Many aspects of the processes of growth and death of cells remain unknown despite their vital importance and the substantial amount of study devoted to these subjects. Since the work of Corper,[1] in 1912, the chemical changes associated with death and necrosis of tissues have received relatively little attention. Although there have been some observations of changes of a few components during the process of tissue death,[2-12] no comprehensive chemical studies with modern techniques and few histochemical observations have been made. A series of studies on necrosis in liver and other tissues has therefore been undertaken in this laboratory.

Liver was selected as the initial tissue for this work because of the substantial amount of information available in this [13-23] and other laboratories [24] on its behavior under different conditions of growth. In general, the liver is considered to consist predominantly of hepatic parenchymal cells and therefore to be suitable for chemical studies. However, observations have shown that 35% or more of the cells of normal mouse and rat liver [23] are of other types. In abnormal livers the percentage of other cell types is much higher.

Most of the studies of necrosis of liver produced by carbon tetrachloride or by diet reveal a variable, diverse response of the tissue, comprising a mixture of dead, dying, regenerating, and otherwise metabolically altered cells, which renders precise interpretation of the results difficult. In this initial study, therefore, a simple procedure was followed which would produce relatively uniform death of all cells uncomplicated by regeneration or other processes. Observations on the chemical changes in mouse liver during necrosis or autolysis in vitro have been reported.[25-27] In this paper the histological and histochemical findings on the same tissues will be presented.

Materials and Methods

Strain A male mice 10 to 14 weeks of age were used in this experiment. In order to maintain a more stable metabolic state of the liver, all mice were deprived of food, but not water, for 18 hours prior to killing by decapitation. After drainage of the blood and removal of the gallbladder, the livers were used for the chemical and histochemical studies. A sterile technique was employed in order to eliminate bacterial growth in the experimental tissues. In each experiment, pieces of liver 3 to 5 mm. in diameter from each of 12 animals were placed in two separate groups in the same sealed weighing bottle for chemical and histochemical studies. Several layers of filter paper, saturated with sterile saline to prevent desiccation of the tissue, were placed on the bottom of the weighing bottle. The pieces of liver were placed on a layer of glass rods above the filter paper; thus the tissue did not come in contact with the paper or any fluid which oozed from the tissues. After 1, 6, 24, 30, 48, and 72 hours of incubation at 37 C the tissues were removed for immediate chemical analysis and fixation. Liver tissues for control observations were also taken from each animal at the beginning of each experiment. Both the experimental and the control tissues were fixed in chilled acetone, 10% formalin, and Stieve fluid [28] and frozen-dried.[29]

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For acetone fixation, pieces of tissue 1 mm. thick were placed in small bottles of chilled absolute acetone and agitated gently with glass beads on a variable speed shaker for a period of 24 hours at 6 C. The cold acetone was changed twice in the initial 4 hours and again twice during the next 20 hours. The tissues were then brought to room temperature for one-half hour and were given two changes of chloroform for one hour and finally infiltrated with paraffin at 56 C for one hour. The tissue blocks and sections were stored at 6 C until needed.

For the demonstration of enzymes, the frozen-dried and acetone-fixed liver was sectioned at 4μ, deparaffinized through two changes of xylene, and hydrated through a graded series of acetone solutions. For staining of alkaline phosphatase and esterase, after a second change of anhydrous acetone, the slides were coated with 1% celloidin, then transferred directly to water. After being washed briefly in distilled water, the slides were immersed in the incubating media at 37 C for a predetermined optimum time, which was kept constant for each enzyme throughout the entire experiment. For demonstration of esterase [89] in sorbitan monostearate (Tween 60) medium at pH 7.2, frozen-dried tissues were incubated for 18 hours and acetone-fixed tissues, for 21 hours. For demonstration of alkaline phosphatase [89] in glycerophosphate medium at pH 9.4, frozen-dried tissues were incubated two and one-half hours and acetone-fixed tissues for five and one-half hours. For demonstration of acid phosphatases,[81] nondeparaffinized slides [77,82] were placed directly into Gomori's substrate for one and one-fourth hours, while frozen-dried tissues and acetone-fixed tissues were immersed for four and five hours, respectively. The control slides for these techniques were treated in the same manner, although the substrate was omitted from the incubating mixture. For comparison with the sorbitan monostearate technique the azo dye technique,[83,84] wherein α-naphthyl acetate and diazo red RC were used in the medium, was also employed for demonstration of esterases.

Frozen-dried sections were deparaffinized in two changes of xylene, air-dried, and stained with pyronin Y and methyl green [85] for demonstration of nucleic acids. To remove ribonucleic acid, the control slides [86] were immersed in crystalline ribonuclease at a concentration of 0.1 mg. per milliliter of McIlvaine's buffer at room temperature for 30 minutes. Tissues fixed in Stieve fluid were stained with the Feulgen reaction [87] and counterstained with fast green for demonstration of deoxypentose nucleic acid.

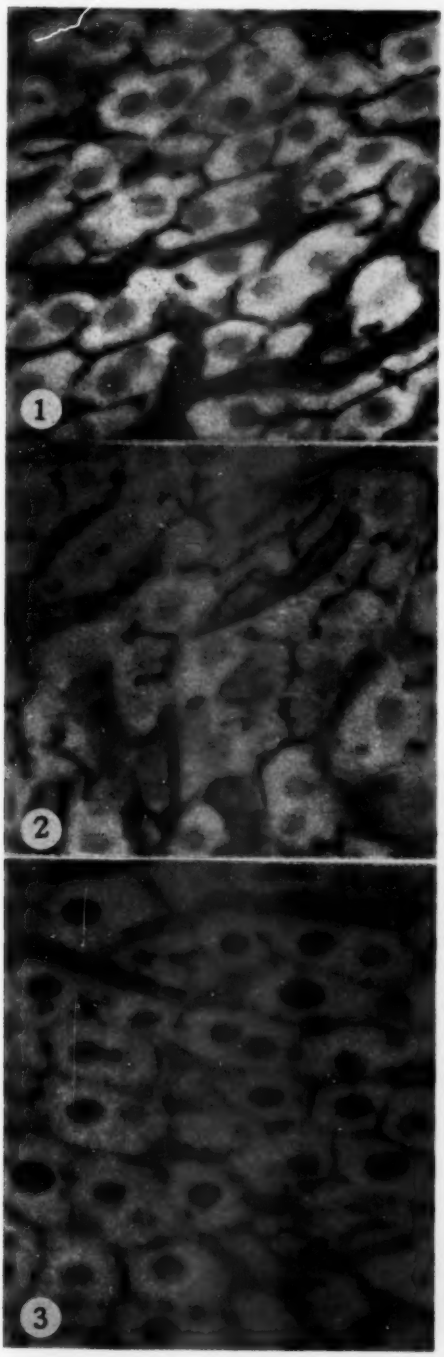
For general histological examination, both frozen-dried and tissues fixed in Stieve fluid were stained with hematoxylin and eosin. Control tissues were observed in each series of experiments.

Observations

General Histological Changes.—After one hour of incubation at 37 C in vitro, the liver cells showed little morphological changes in frozen-dried tissues stained with hematoxylin and eosin, aside from a slight hyperchromatism of the nuclei, especially of the Kupffer cells and endothelial cells. In tissues fixed in Stieve fluid and stained with hematoxylin and eosin, a dilation of the sinusoids throughout the tissue and some Disse spaces between the sinusoidal lining and parenchymal cells were noted. This difference in sinusoidal spaces in tissue fixed in Stieve fluid and by freeze-drying was interpreted as being related to the nature of the two fixation processes. Freeze-drying preserves more of the materials in and about the sinusoids, causing the latter to be less evident, whereas more differential shrinkage of tissue constituents and dissolution of materials makes more prominent sinusoidal and Disse spaces in tissue fixed in Stieve fluid and subsequently dehydrated.

Early pyknotic changes of the hepatic parenchymal cells began to show after six hours of incubation. The nuclei of endothelial cells and most of the Kupffer cells were distinctly hyperchromatic. The cytoplasm of hepatic parenchyma was still homogenous and stained paler than the control tissue. The granular material in the cytoplasm was somewhat finer than that of the control tissue.

Marked changes occurred after 24 hours of incubation. In frozen-dried tissue, the necrosis of the hepatic cells was sometimes more advanced in the center of the block, where karyolysis and karyorrhexis were prominent. Many nuclei of endothelial cells were hyperchromatic throughout the pieces of tissue. Most of the nuclei of the hepatic parenchymal cells had lost much of their chromatin; usually, however, a few pyknotic nuclei were found along the outer margin of the piece. Fragmented nuclear material was scattered in the sinusoids. In many areas the sinusoids appeared dilated. Some of the cells in the periphery of the tissue



became swollen with foamy less basophilic cytoplasm. Generally, most of these cells still stained, although in some pieces they stained faintly. These microscopic findings varied slightly from one experiment to another and from one piece of tissue to another within the same experiment. On the whole, the appearance of the tissue fixed in Stieve fluid was similar to that which was frozen-dried. After 30 hours of incubation, tissues showed decreased ability to stain with hematoxylin and eosin and some areas became completely necrotic.

The liver was completely necrotic after 48 to 72 hours of incubation. In some areas only traces of nuclear material staining were apparent in the cells; otherwise, the nuclei appeared lysed. The hepatic cells were partially separated or isolated from each other.

Alkaline Phosphatase.—In normal livers (Fig. 1), this enzyme was localized chiefly at the periphery of bile canaliculi in Kupffer and endothelial cells and within the sinusoids. A strong reaction was usually found in the portal area, particularly in the bile ducts, in venous and arterial endothelium, and in connective tissue surrounding the bile duct. The frozen-dried tissues usually exhibited a darker and more precisely defined localization and less nuclear reaction than the acetone-fixed tissues. After one hour of incubation, the tissue began to show a change of pattern of enzyme activity (Fig. 2). Almost invariably, dark course granules adhered to the bile canaliculi, presenting a rough appearance. In contrast, in normal tissues the canaliculi were always stained intensely and were uniform in contour. In general, after one hour of incubation, about the same amount of enzymatic reaction was observed in the experimental tissues as in the control tissue, although the nuclei and

Plate I

Alkaline phosphatase metal precipitate reaction in mouse liver prepared by freeze-drying. Normal liver in Figure 1 shows strong reaction in bile canaliculi and sinusoids and slight nuclear reaction. After one hour of incubation, granules are noted along the bile capillaries (Fig. 2), whereas by six hours there is a general loss of enzyme and granular deposits on bile canaliculi with increased nuclear staining (Fig. 3); × 520.

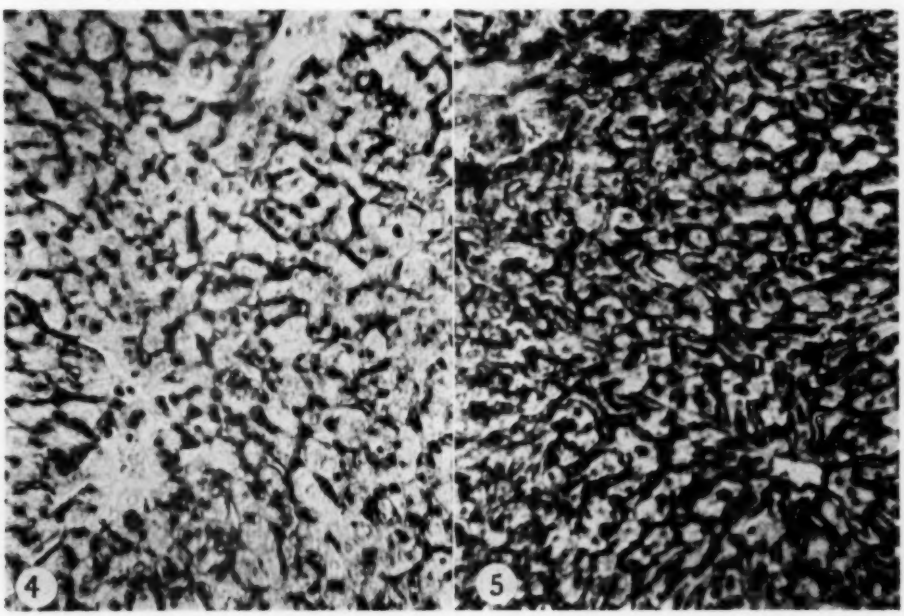
nucleoli had slightly more in the former. After six hours of incubation, however, the tissues began to show an over-all loss of activity (Fig. 3). The bile canaliculi, Kupffer cells, and sinusoids were stained faintly, but the nuclei and nucleoli of the parenchymal cells showed a marked increase of activity. There was usually a strong reaction in vascular endothelium. A few of the tissues showed a diffuse reaction, a homogeneous gray opaque appearance. After 24 to 30 hours of incubation, a slight activity could be inconsistently demonstrated in nuclei, in a few bile canaliculi, and in sinusoids; otherwise, there was only a weak diffuse activity. The completely necrotic tissues, after 48 to 72 hours of incubation, showed negligible or slight diffuse enzymatic activity with only occasional weak reaction in nuclei and sinusoids.

Acid Phosphatase.—Tissues prepared by freeze-drying and acetone fixation did not react for acid phosphatase in the same manner in these experiments. Normal acid phosphatase activity was localized in the lumina and walls of sinusoids, in the cytoplasm of leukocytes, in Kupffer cells, in hepatic parenchymal cells, in bile duct epithelium, and in endothelium. In frozen-dried tissues, there was no marked decrease during the first six hours of incubation. After 24 to 30 hours, the cytoplasm of hepatic parenchymal cells lost some of the enzymatic activity, though the sinusoidal contents and Kupffer and endothelial cells still retained considerable activity. The enzyme was further decreased as the necrosis progressed; however, even after 72 hours of incubation, an appreciable enzymatic activity persisted in endothelium.

In acetone-fixed tissue, in all series of experiments, enzymatic activity in the nuclei and cytoplasm of hepatic parenchymal cells seemed to increase slightly after the tissue had been incubated for one to six hours. The intensity of the reaction increased markedly after 24 hours of incubation (Figs. 4 and 5), then decreased gradually at 48 and 72 hours.

Plate II

Acid phosphatase metal precipitate reaction in mouse liver fixed in acetone. Tissue left in the incubator 24 hours shows much more reaction (Fig. 5) than normal liver (Fig. 4); × 280.



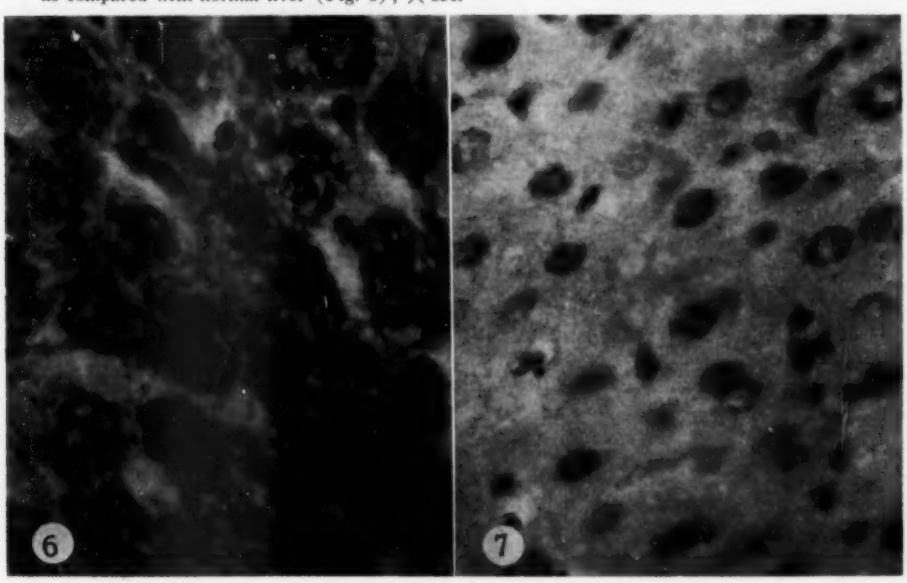
Even in 72-hour tissue the reaction was stronger than in the control tissues.

Esterases.—Following both the freeze-drying and the acetone fixation of the tissues, the nonspecific esterase enzymes were found localized in the cytoplasm of the hepatic cells and bile duct epithelial cells by the sorbitan monostearate [29] and azo dye [33,34] techniques. In the control tissue, usually the reaction was slightly stronger in the central areas of the lobules than in the periportal zone. Some variations were observed, however, in tissues of different experiments. No conspicuous change in the intensity of enzyme activity was noticed in tissues after one or six hours of incubation. After 24 to 30 hours, the peripheral and, at times, the central area of the tissue began to lose some activity. Further, some of the enzymes tended to diffuse from the cell cytoplasm into the sinusoidal spaces. An increasing diffuse reaction and a diminution of the focal enzyme activity appeared in the tissues in later stages of necrosis. Large lumps of metal precipitate usually were scattered in the tissue after 48 hours in the incubator; this precipitate did not appear in slides treated with azo dye technique. Considerable activity remained in the 72-hour necrotic tissues.

Nucleic Acids.—At one hour the slides of tissue fixed in Stieve fluid, stained by the Feulgen reaction, and counterstained with fast green confirmed the previously described swelling of sinusoidal spaces with shrinkage of sinusoidal lining and evident space of Disse, as well as slight pyknosis of some of the nuclei of Kupffer cells. At 24 and 30 hours of incubation, some areas of the liver showed no staining for deoxypentose nucleic acid; in other areas the hepatic parenchymal cells showed an increased tendency to stain with fast green and Feulgen-positive material was relatively absent at the nuclear membrane but persisted in the chromatin masses within the nucleus. There was some Feulgen staining of nuclear debris within the sinusoidal spaces. By 48 hours the Feulgen reaction was negative and only an

Plate III

Pyronin and methyl green stain on mouse liver prepared by freeze-drying. The pentose nucleic acid in the cytoplasm is markedly decreased after 24 hours of incubation (Fig. 7), as compared with normal liver (Fig. 6); × 850.



occasional nucleus remained stained with fast green.

There was no noticeable difference of nuclear staining with methyl green between the control tissues and the tissues incubated for one or six hours. Only a slight decrease of pyronin, and presumably pentose nucleic acid, in cytoplasm and nucleoli was found after six hours of incubation. Pentose nucleic acid was sharply decreased after 24 hours of incubation (Figs. 6 and 7), but methyl green stain remained intense in some of the nuclei. After 30 hours little pentose nucleic acid was observed in necrotic tissue, while nuclear reaction with methyl green persisted. At 48 hours of incubation, pyronin stain had disappeared almost entirely, and only traces of methyl green were found in some nuclei. Staining for both types of nucleic acid usually vanished completely in tissue undergoing necrosis for 72 hours.

Comment

Frozen-dried tissues, which are superior in many respects to tissues fixed by protein denaturation with chemical agents,[38] were used extensively for histological and histochemical observations in this investigation. Generally, the tissues stained with hematoxylin and eosin were only slightly altered morphologically after one or six hours of incubation. Necrosis of the central part of the tissue took place within 24 hours. Usually necrosis was essentially complete within 48 hours.

Since both histochemical staining and analytical biochemical methods have their limitations,[39] more accurate observations on the localization and quantization of chemical constituents of tissues can usually be obtained by a judicious combination of both methods. The potential loss of lyoenzymes as contrasted to desmoenzymes[40] in these experiments is unknown.

Chemical data on these same tissues[25] at 6, 24, 48, and 72 hours showed a steady decline of percentage activity figures as compared with controls of 98%, 66%, 32%, and 20% for alkaline phosphatase; of 75%, 37%, 17%, and 14% for acid phosphatase, and of 94%, 54%, 22%, and 12% for esterase. The histochemical findings, while showing some changes in different sites, generally agreed with the chemical data; some points, however, are of special interest.

The histochemical preparations suggest that some of the 32% and 20% of alkaline phosphatase activity remaining at 48 and 72 hours, respectively, as shown by chemical studies, may be attributed to the diffuse reaction. At this time the activity was no longer confined to the normal sites. The histochemical slides showed a definite increase of granular deposits along the bile canaliculi after one hour of incubation. These deposits might be related to a slightly increased activity of alkaline phosphatase of questionable significance as determined chemically.[25]

The specificity of Gomori's acid phosphatase technique has been questioned.[41,42] By using frozen-dried tissue and nondeparaffinized slides and by taking all the necessary precautions recommended by Gomori,[30,43] one can often obtain a good picture of the enzyme localization in the normal tissues. In necrotic liver, however, the section of frozen-dried tissues did not always show a corresponding reduction of enzyme activity, as indicated by the chemical data. Further, the intensity of reaction in necrotic tissue fixed in acetone was definitely increased. This is contradictory to the chemical findings and to theoretical principles. Since one would not expect synthesis of an enzyme in dying tissue, increased enzyme activity is not anticipated. On the other hand, when slides were incubated in a medium without substrate, there was no reaction on the slides. Also, the location and appearance of the deposits suggests that the enzymatic reaction is a true positive reaction. This apparent increase in acid phosphatase may be related to the process of fixation of these necrotic tissues with acetone by mechanisms unknown to us. Obviously, the reason for this discrepancy in results needs further study.

The changes in nucleic acid content appeared to be similar in the chemical and histochemical observations, especially the sharp drop of pentose nucleic acid after 24 hours. The chemical data on these tissues at 6, 24, 30, and 48 hours showed remaining percentages of pentose and deoxypentose nucleic acid, respectively, of 76%, 32%, 11%, and 7%, and 94%, 50%, 29%, and 12%. The fact that a more complete loss of nucleic acids was observed histochemically than chemically in the 48- and 72-hour tissues is explained by the probable lack of absolute specificity and quantitative accuracy of such histochemical staining methods and loss of substances during fixation, paraffin embedding, paraffin removal, and hydration.[38] Other instances of incomplete agreement of results of histochemical and chemical determinations on pentose nucleic acids have been reported.[15,44,45]

The fact that chemical and histochemical techniques for deoxypentose nucleic acid have in part different bases for their reaction could explain some of the variations in results if different parts of the nucleic acid molecules were affected during various states of the process of necrosis. Chemical measurements were made by analyses for ribonucleic acid and deoxyribonucleic acid on the nucleic acid fractions obtained by the method of Schneider.[46] The orcinal reaction[47] determines the pentose associated with ribonucleic acid purines only. The diphenylamine reaction of Dische[48] and the Feulgen technique[49,50] determine the aldehyde groups of deoxypentose associated with the purines of deoxypentose nucleic acid. The sum of these chemical reactions for deoxypentose and ribonucleic acids was checked against the total nucleic acid as determined by ultraviolet absorption analysis; this latter depends upon the absorption of the alternating and resonating double-bond structures of the purines and pyrimidines in both types of nucleic acid molecules. The methyl green reaction may be related to the presence of phosphate radical in polymerized deoxyribonucleic acid.[51]

Summary

Histological and histochemical changes were studied in pieces of mouse liver incubated at 37 C under sterile conditions at intervals as long as 72 hours. As visualized with hematoxylin and eosin staining, necrosis often began in the central part of the tissue at 6 to 24 hours and was essentially complete within 48 hours. In the tissue fixed in Stieve fluid, among the earliest changes were pyknosis of Kupffer and sinusoidal lining cells, dilation of sinusoidal spaces, and appearance of Disse's spaces; these changes began at one hour.

The general reduction of alkaline phosphatase and esterase corresponded to the decreases noted in chemical studies on these same tissues; alkaline phosphatase, however, showed an alteration in distribution after 1 hour of incubation and a weak and diffuse reaction after 30 hours. At that time a third of the enzyme was still present, as determined chemically. The techniques for esterase employing both azo dye and sorbitan monostearate (Tween 60) substrate gave results which were generally similar. A substantial decrease of esterase activity with diffusion of the enzyme was noted after 24 to 30 hours, but appreciable activity remained at 72 hours; only 12% of the esterase was then detected chemically.

The loss of acid phosphatase activity in necrotic liver which had been frozen-dried was not evident by six hours, though 25% loss was observed by chemical determinations. A decrease was observed histochemically, beginning at 24 to 30 hours. In acetone-fixed necrotic tissue, acid phosphatase activity increased, reaching a peak in 24 to 30 hours; even in 72-hour tissue, the reaction was stronger than in the control tissue. This discrepancy between the two types of histochemical studies was not understood by us.

Beginning after six hours of incubation, pentose nucleic acid decreased histochemically in cytoplasm and nucleoli, and the decrease was pronounced at 24 hours; at these times chemical decreases of 24% to 68%

were observed. The histochemical observations on deoxypentose nucleic acid, by use of the Feulgen reaction and methyl green, suggested a more rapid loss in the Feulgen-stained slides. With methyl green a detectable histochemical decrease in the deoxypentose nucleic acid in the nuclei was noted at 24 hours and only slight staining remained at 48 hours; at these times 50% and 12% of the deoxypentose nucleic acid remained as found by chemical measurements. By 24 hours of incubation the Feulgen reaction suggested a complete loss in deoxypentose nucleic acid in some areas of the tissue and a loss of nucleic acid from the nuclear membrane in other areas. The Feulgen reaction was completely negative by 48 hours.

The incubation of liver tissue in vitro is a useful means of studying the chemical changes associated with cell death and necrosis. The histochemical techniques, while less quantitative than chemical methods, provide useful observations on distribution and are helpful when used in conjunction with chemical studies. In addition to early changes in the oxidative enzymes, which started at one hour in the chemical studies, striking early changes were observed histochemically, i. e., the shifting of location of alkaline phosphatase at one hour and the sharp decrease in staining of pentose nucleic acid at six hours.

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REFERENCES

1. Corper, H. J.: Correlation of the Histological and Chemical Changes in the Spleen During Necrosis and Autolysis, J. Exper. Med. 15:429-449, 1912.

2. Abell, M. R., and Beveridge, J. M. R.: Hepatic Necrosis Induced by Dietary Means: II. Biochemical Changes Occurring in the Liver During the Development of Necrosis, Arch. Path. 50:23-35, 1950.

3. Baker, H. deC.: Ischaemic Necrosis in the Rat Liver, J. Path. & Bact. 71:135-143, 1956.

4. Bradley, H. C.: Studies of Autolysis: VIII. The Nature of Autolytic Enzymes, J. Biol. Chem. 52:467-484, 1922.

5. Bradley, H. C.: Autolysis and Atrophy, Physiol. Rev. 18:173-196, 1938.

6. Gallagher, C. H.; Judah, J. D., and Rees, K. R.: Enzyme Changes During Liver Autolysis, J. Path. & Bact. 72:247-256, 1956.

7. Himsworth, H. P., and Glynn, L. E.: The Gross Chemical Changes in the Liver in Dietetic Necrosis, Biochem. J. 39:267-271, 1945.

8. Sevringhaus, E. L.; Koehler, A. E., and Bradley, H. C.: Studies of Autolysis: IX. Hydrogen Ion Concentration in Autolysis, J. Biol. Chem. 57:163-179, 1923.

9. LePage, G. A.: Biological Energy Transformations During Shock as Shown by Tissue Analyses, Am. J. Physiol. 146:267-281, 1946.

10. Luck, J. M.; Eudin, J., and Nimmo, C. C.: Autolytic Changes in the Protein and Amino Acid Content of Liver, J. Biol. Chem. 131:201-209, 1939.

11. Potter, V.; LePage, G. A., and Klug, H. L.: The Assay of Animal Tissues for Respiratory Enzymes: VII. Oxalacetic Acid Oxidation and the Coupled Phosphorylations in Isotonic Homogenates, J. Biol. Chem. 175:619-634, 1948.

12. Sperry, W. M.; Brand, F. C., and Copenhaver, W. M.: The Behavior of Lipids During Autolysis of Liver and Brain, J. Biol. Chem. 144:297-306, 1942.

13. Stowell, R. E.: Nucleic Acids and Cytologic Changes in Regenerating Rat Liver, Arch. Path. 46:164-178, 1948.

14. Stowell, R. E.: Alterations in Nucleic Acids During Hepatoma Formation in Rats Fed p-Dimethylaminoazobenzene, Cancer 2:121-131, 1949.

15. Stowell, R. E., and Lee, C. S.: Histochemical Studies of Mouse Liver After Single Feeding of Carbon Tetrachloride, Arch. Path. 50:519-537, 1950.

16. Stowell, R. E.: Lee, C. S.; Tsuboi, K. K., and Villasana, A.: Histochemical and Microchemical Changes in Experimental Cirrhosis and Hepatoma Formation in Mice by Carbon Tetrachloride, Cancer Res. 11:345-354, 1951.

17. Tsuboi, K. K., and Stowell, R. E.: Enzyme Alterations Associated with Mouse Liver Degeneration and Regeneration After Single Carbon Tetrachloride Feeding, Cancer Res. 11:221-228, 1951.

18. Tsuboi, K. K.; Stowell, R. E., and Lee, C. S.: Chemical Alterations Induced in Mouse Liver Following a Single Feeding of Carbon Tetrachloride, Cancer Res. 11:87-93, 1951.

19. Tsuboi, K. K.; Yokoyama, H. O.; Stowell, R. E., and Wilson, M. E.: The Chemical Composition of Regenerating Mouse Liver, Arch. Biochem. 48:275-292, 1954.

20. Wilson, M. E., and Stowell, R. E.: Cytological Changes Following Roentgen Irradiation of the Liver in Mice, J. Nat. Cancer Inst. 13:1123-1137, 1953.

21. Wilson, M. E.; Stowell, R. E.; Yokoyama, H. O., and Tsuboi, K. K.: Cytological Changes in Regenerating Mouse Liver, Cancer Res. 13:86-92, 1953.

22. Yokoyama, H. O.; Tsuboi, K. K.; Wilson, M. E., and Stowell, R. E.: Histochemical Studies on Regenerating Mouse Liver, Lab. Invest. 2:91-108, 1953.

23. Yokoyama, H. O.; Wilson, M. E.; Tsuboi, K. K., and Stowell, R. E.: Regeneration of Mouse Liver After Partial Hepatectomy, Cancer Res. 13:80-85, 1953.

24. Himsworth, H. P.: Lectures on the Liver and Its Diseases, Cambridge, Mass., Harvard University Press, 1948.

25. Berenbom, M.; Chang, P. I.; Betz, H., and Stowell, R. E.: Chemical and Enzymatic Changes Associated with Mouse Liver Necrosis in Vitro, Cancer Res. 15:1-5, 1955.

26. Berenbom, M.; Chang, P. I., and Stowell, R. E.: Changes in Mouse Liver Undergoing Necrosis in Vivo, Lab. Invest. 4:315-323, 1955.

27. Stowell, R. E.; Berenbom, M., and Chang, P. I.: Biochemical and Histochemical Studies of in Vivo and in Vitro Necrosis of Liver Tissue, Am. J. Path. 30:618, 1954.

28. Stowell, R. E.: A Modified Freezing-Drying Apparatus for Tissues, Stain Technol. 26:105-108, 1951.

29. Gomori, G.: The Microtechnical Demonstration of Sites of Lipase Activity, Proc. Soc. Exper. Biol. & Med. 58:362-364, 1945.

30. Gomori, G.: Microscopic Histochemistry: Principles and Practice, Chicago, University of Chicago Press, 1952.

31. Gomori, G.: An Improved Histochemical Technic for Acid Phosphatase, Stain Technol. 25:81-85, 1950.

32. Goetsch, J. B., and Reynolds, P. M.: Obtaining Uniform Results in Histochemical Technic for Acid Phosphatase, Stain Technol. 26:145-151, 1951.

33. Nachlas, M. M., and Seligman, A. M.: The Histochemical Demonstration of Esterase, J. Nat. Cancer Inst. 9:415-425, 1949.

34. Nachlas, M. M., and Seligman, A. M.: The Comparative Distribution of Esterase in the Tissues of 5 Mammals by a Histochemical Technique, Anat. Rec. 105:677-695, 1949.

35. Taft, E. B.: The Problems of a Standardized Technic for the Methyl-Green-Pyronin Stain, Stain Technol. 26:205-212, 1951.

36. Stowell, R. E., and Zorzoli, A.: The Action of Ribonuclease on Fixed Tissues, Stain Technol. 22:51-61, 1947.

37. Stowell, R. E.: Feulgen Reaction for Thymonucleic Acid, Stain Technol. 20:45-58, 1945.

38. Berenbom, M.; Yokoyama, H. O., and Stowell, R. E.: Chemical and Enzymatic Changes in Liver Following Freezing-Drying and Acetone Fixation, Proc. Soc. Exper. Biol. & Med. 81:125-128, 1952.

39. Stowell, R. E.: Use of Histochemical and Cytochemical Technics in Problems in Pathology, Lab. Invest. 1:210-230, 1952.

40. Nachlas, M. M.; Prinn, W., and Seligman, A. M.: Quantitative Estimation of Lyo- and Desmo-Enzymes in Tissue Sections With and Without Fixation, J. Biophys. & Biochem. Cytol. 2:487-502, 1956.

41. Palade, G. E.: Intracellular Distribution of Acid Phosphatase in Rat Liver Cells, Arch. Biochem. 30:144-158, 1951.

42. Palade, G. E.: Intracellular Localization of Acid Phosphatase: A Comparative Study of Biochemical and Histochemical Methods, J. Exper. Med. 94:535-548, 1951.

43. Gomori, G.: Sources of Error in Enzymatic Histochemistry, J. Lab. & Clin. Med. 35:802-809, 1950.

44. Farber, E.; Koch-Weser, D.; Szanto, P. B., and Popper, H.: Correlation Between Cytoplasmic Basophilia and the Nucleic Acid Content of the Liver, Arch. Path. 51:399-422, 1951.

45. Koch-Weser, D.; Farber, E., and Popper, H.: Fatty Liver With and Without Necrosis: Histological and Biochemical Investigations, Arch. Path. 51:498-509, 1951.

46. Schneider, W. C.: Phosphorus Compounds in Animal Tissues: I. Extraction and Estimation of Desoxypentosenucleic Acid and of Pentose Nucleic Acid, J. Biol. Chem. 161:293-303, 1945.

47. Brown, A. H.: Determination of Pentose in the Presence of Large Quantities of Glucose, Arch. Biochem. 11:269-278, 1946.

48. Dische, Z.: Some New Characteristic Color Tests for Thymonucleic Acid and a Microchemical Method for Determining the Same in Animal Organs by Means of These Tests, Mikrochemie 8: 4-32, 1930.

49. Overend, W. G., and Stacey, M.: Mechanism of the Feulgen Nucleal Reaction, Nature, London 163:538-540, 1949.

50. Tamm, C.; Hodes, M. E., and Chargaff, E.: The Formation of Apurinic Acid from the Desoxyribonucleic Acid of Calf Thymus, J. Biol. Chem. 195:49-63, 1952.

51. Kurnick, N. B., and Foster, M.: Methyl Green: III. Reaction with Desoxyribonucleic Acid, Stoichiometry, and Behavior of the Reaction Product, J. Gen. Physiol. 34:147-159, 1950.

Anomalous Band of the Left Atrium

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Anomalous bands inside the left auricle are exceedingly rare. The purpose of this paper is to report briefly such a case.

Report of a Case

A 62-year-old white man had numerous hospital admissions since 1955, when he was hospitalized because of shortness of breath on exertion and because of a cough, which had started approximately one year previously. Since 1955 the patient had been taking digitalis, and he had also received aminophylline, penicillin, and potassium iodide. He had had several phlebotomies. He had established diagnoses of chronic bronchitis, bronchial asthma, pulmonary emphysema, and chronic cor pulmonale. His final admission was on Sept. 1, 1957.

Physical examination revealed an extremely cyanotic white man complaining of severe dyspnea and cough. His face showed a rubor cyanosis. The chest showed an increase of the anteroposterior diameter. There were numerous coarse rhonchi and wheezes present throughout both lung fields. The heart size was within normal limits. The rate was rapid but regular. No murmurs were heard. The pulmonic second sound was accentuated. The blood pressure was 120/70. X-ray of the chest showed pulmonary emphysema and a normal heart shadow. ECG showed tall, peaked P waves and was compatible with cor pulmonale. The temperature was 103.6 F. The hematocrit was 50% on one occasion and 51% on another.

Postmortem Examination.—The lungs were typical of pulmonary emphysema and will not be discussed here. The kidneys were normal. The description is limited to the heart.

The heart weighed 440 gm. The valves were intact and of normal size. The left ventricle was 17 mm. in thickness, and the right ventricle, 8 mm. in its thickest part. The myocardium was firm in consistency. Mural thrombi were not found in the auricular appendages. The atria were of normal size. The coronary arteries showed moderate atheromatous changes. The foramen ovale was closed.

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From the Medical and Pathology Services of the Veterans' Administration Center.

Anomalous band of the left atrium.

Located in the left atrium and extending from the anterior to the posterior wall there was a pale gray band of tissue, measuring 3.8 cm. in length and 1.5 mm. in diameter. The surface of this band was smooth and was covered by endocardium. The band was attached to the atrium just distal to the openings of the right pulmonary veins at one end and just distal to the openings of the left pulmonary veins at the opposite end. Both attachments were flat and somewhat fan-shaped and measured 8 mm. across. Centrally, this band became thin and rounded, cord-like, measuring 1.5 mm. in diameter at its narrowest point.

Comment

Anomalous bands of the right atrium occur not infrequently. According to Helwig, the incidence is about 1.5% of all necropsies [1]; the incidence is 2%-3% of all cases of congenital heart disease, according to Friedberg.[2] The bands are remnants of the valvula of the sinus venosus and extend

from the Thebesian to the Eustachian valve, the so-called Chiari's network.[2]

However, anomalous bands in the left atrium are rare. Helwig, in 1932, in reviewing anomalous bands, cords, strands, or reticula of the left atrium, found in the world literature 17 instances of cords and 15 cases of septums and diaphragms and added 1 case of his own, which was a fenestrated hammock-like structure in the left atrium.[1] In 1954 Pedersen and Therkelsen[3] reviewed the literature and reported that 23 cases of abnormal cords, bands, or reticula of the left atrium had been found. Of these reported cases, one attachment of the cord was always in the neighborhood of the fossa ovalis, with the other end inserted into the atrium or at the mitral valve. Kjellberg et al. state that "in rare cases strands may also be found in the left atrium; they generally originate from the foramen ovale region and represent rudimentary equivalents of the septum formations which contribute to division of the atrium," and their illustrations show a band of the left atrium running from the foramen ovale to the mitral valve.[4] We have found only one case, reported by McNamara et al. in 1947, of an anomalous band in the left atrium similiar to that of our case.[5] Their case also had concomitant polycystic kidney disease, which was not present in our case.

Embryologically the presence of those cords and bands is explained in two ways: Either they represent remnants of the septum primum or those anomalous bands are considered to be a developmental anomaly of the embryonal common pulmonary vein, possibly an arrested development during its stage of absorption into the walls of the auricle.[6-8] Probably those cases in which the strand arises in the region of the foramen ovale and inserts into the mitral valve belong to the first category. In our case the foramen ovale was intact, and the band was inserted above the foramen ovale; it did not fuse with the mitral valve but was inserted into the free wall of the left atrium. We think that it can be explained as a remnant of an abnormal development of the common pulmonary vein.

Whether this band could have interfered with a normal functioning of the left auricle is questionable. This patient did not have auricular fibrillation or any other arrhythmia. He had been fluoroscoped frequently, and no abnormalities in the expansion of the left auricle were noted. Neither were there thrombi found in the left auricle or left auricular appendage. This band did not produce any murmur.

Summary

A case of an anomalous band of the left atrium is reported. No other abnormalities were present. It was an incidental finding and was asymptomatic. The rarity of anomalous bands is stressed.

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REFERENCES

1. Helwig, F. C.: Fenestrated Hammock-like Structure in the Left Cardiac Atrium, Arch. Path. 15:8, 1933.

2. Friedberg, C. K.: Diseases of the Heart, Ed. 2, Philadelphia, W. B. Saunders Company, 1956, p. 792.

3. Pedersen, A. and Therkelsen, F.: Cor Triatriatum: A Rare Malformation of the Heart, Probably Amenable to Surgery, Am. Heart J. 47:676, 1954.

4. Kjellberg, S. R.; Mannheimer, E.; Rudhe, U., and Jonsson, B.: Diagnosis of Congenital Heart Disease, Chicago, The Year Book Publishers, Inc., 1955, pp. 28-29.

5. McNamara, W. L.; Baker, L. A., and Costich, K. J.: Asymptomatic Congenital Anomaly of the Heart: Congenital Muscular Cord Bridging Walls of Auricle Above Center of Mitral Valve, Am. Heart J. 34:288, 1947.

6. Loeffler, E.: Unusual Malformation of the Left Atrium: Pulmonary Sinus, Arch. Path. 48: 371, 1949.

7. Edwards, J. E.; DuShane, J. W.; Alcott, D. L., and Burchell, H. B.: Thoracic Venous Anomalies, A. M. A. Arch. Path. 51:446, 1951.

8. Parsons, C. G.: Cor Triatriatum: Concerning the Nature of an Anomalous Septum in the Left Auricle, Brit. Heart J. 12:327, 1950.

Absence of the Transverse Aortic Arch

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Complete absence of the transverse aortic arch is said to be a rare congenital defect, since only 21 cases have been reported. However, during the past 10 years, six cases of this anomaly have been seen in the pathology department of Cleveland City Hospital. This suggests that the occurrence of absent transverse aortic arch may be more frequent than previously suspected. This paper describes these six cases, reviews the literature to the present time, and analyzes the associated cardiovascular defects and the embryologic factors responsible for this lesion.

Report of Cases

CASE 1.—A full-term boy weighed 7 lb. 1 oz. at birth. At the age of 6 days it was noted that he was breathing rapidly. He was fretful and slept poorly. The following day the child was feverish, and clonic movements of the right arm and leg were observed.

Physical Examination.—The infant was stuporous and did not respond to noxious stimuli. No cyanosis was noted. There was a cleft in the soft palate. Tissue turgor was poor, and the anterior fontanelle was firm but not bulging. There was slightly decreased resonance on percussion over the posterior lateral lung fields. Breath sounds were depressed over both bases. No rales were heard. The heart did not appear enlarged, and no murmurs were heard. The heart rate was rapid but regular. A mass which moved with respiration was noted 2 cm. below the right costal margin and in the epigastric region. Chvostek's sign was present bilaterally. Tendon reflexes were hypoactive throughout.

Laboratory Data.—The red blood cell count was 4,500 000, and white blood cell count, 16,400. The blood calcium and phosphorus were 7.4 mg. and 13.6 mg. per 100 cc., respectively.

Hospital Course.—The infant died 13½ hours after admission. A postmortem lumbar puncture showed xanthochromic fluid, a 4+ Pandy reaction, and 6 white cells.

Postmortem Findings.—The heart was enlarged and weighed 32 gm. (normal weight, 18 gm.). It was of the usual shape and in the normal position. The wall of the right atrium was slightly increased in thickness, and that of the right ventricle measured 3 mm., the same as the left ventricle. The columnae carneae were rounded, and the pectinate and papillary muscles were not remarkable. The foramen ovale was patent and measured 0.6 cm. in diameter. There was an oblong opening in the membranous portion of the interventricular septum, measuring 8×3 mm. The tricuspid valve was not remarkable. The pulmonic valve was composed of the usual three cusps, and the pulmonary conus immediately above it was enlarged and measured 3 cm. in circumference. The aortic ring measured 1.2 cm. in circumference, and the valve was bicuspid. The mitral valve was normal. The coronary arteries arose just above

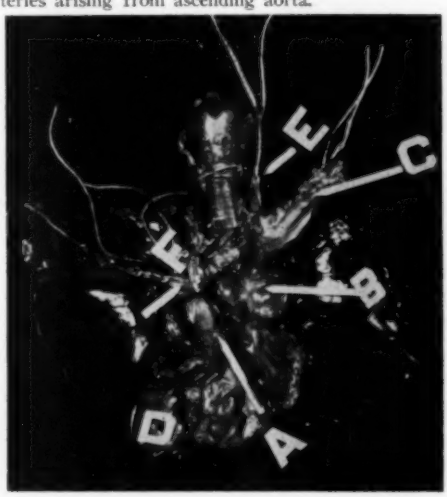

Fig. 1. (Case 1).—*A*, hypoplastic aorta. *B*, pulmonary artery. Note wrinkled portion, representing the persistent ductus arteriosus, distal to branches of pulmonary artery. *C*, descending aorta arising from ductus arteriosus. *D*, right ventricle. *E*, left subclavian artery arising distal to ductus. *F*, right innominate and left common carotid arteries arising from ascending aorta.

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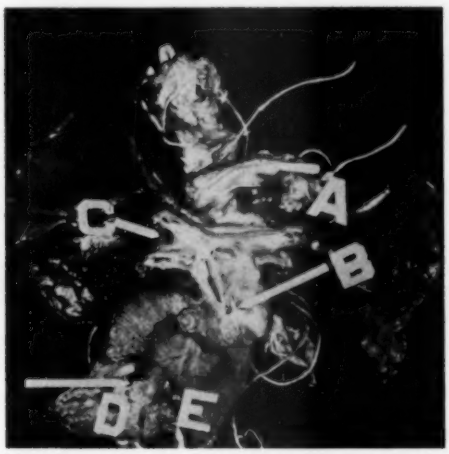

Fig. 2 (Case 1).—*A*, descending aorta. *B*, atretic mitral orifice. *C*, left pulmonary veins. *D*, defective interventricular septum. *E*, site of left ventricle.

the anterior cusp of the aortic valve and were not remarkable.

The ascending aorta was hypoplastic and measured 1.2 cm. in circumference. At a distance of 2.4 cm. from the aortic valve it bifurcated to form the innominate and left common carotid arteries. The aortic arch was absent. The pulmonary trunk arose from the right ventricle and arched to the right and continued as the descending aorta along the right anterior surface of the vertebral column. The pulmonary trunk in the arched portion gave off four vessels. The left pulmonary artery was the first branch and arose 1.5 cm. from the pulmonic valve. The left subclavian artery arose 0.2 cm. distal to this, followed by the right pulmonary artery, which originated 3.8 cm. from the pulmonic valve in the arched portion connecting the pulmonary conus and the descending aorta. The right subclavian arose 0.8 cm. distal to the origin of the right pulmonary artery. The pulmonary trunk then continued as the descending aorta. The vena cavae lay in the usual position.

Summary of Postmortem Findings: (1) absence of aortic arch; (2) hypoplasia of ascending aorta; (3) bicuspid aortic valve; (4) patent foramen ovale; (5) ventricular septal defect; (6) right-sided descending aorta (persistent right ductus arteriosus); (7) anomalous origins of right and left subclavian arteries; (8) subdural hematoma, left parietal; (9) subarachnoid hemorhage, left parietal; (10) focal atelectasis; (11) acute passive hyperemia of all viscera.

Case 2.—A boy weighing 6 lb. 2 oz. was born after a normal pregnancy. He required resuscitation for about 20 minutes after a low forceps delivery. Respirations were normal after resuscitation.

Physical Examination.—The color of the skin and mucous membranes was good, except for the legs, which were cold and blue. The lungs were clear to auscultation and percussion, but respirations were shallow. A faint systolic murmur was heard at the base. He died 51 hours after birth in spite of supportive therapy.

Postmortem Findings.—The heart was of the usual size. The superior and inferior vena cavae entered the right atrium in the usual manner, and the foramen ovale was patent. The tricuspid valve was not remarkable. There was a 0.6 cm. defect in the membranous portion of the interventricular septum. The caliber of the pulmonary artery was larger than that of the aorta, and it arose from the right ventricle. This vessel gave origin to both pulmonary arteries and to the left subclavian artery and continued as the thoracic and abdominal aorta. The pulmonary artery was 2 cm. in circumference and had three normal valve cusps. There was, however, a moderate narrowing and slight intimal irregularity of the pulmonary trunk at the usual site for the ductus arteriosus. The pulmonary veins emptied into the left atrium. The mitral valve was not remarkable. The left and right ventricles measured 3 mm. in thickness. The aorta arose from the left ventricle and was one-half the circumference of the pulmonary trunk. The aortic valve was bicuspid. The ascending aorta branched into the innominate and the left common carotid arteries. The origins of the coronary ostia were normal.

Summary of Postmortem Findings: (1) absence of the transverse aortic arch; (2) hypoplasia of the ascending aorta; (3) biscuspid aortic valve; (4) interventricular septal defect; (5) patent foramen ovale; (6) anomalous origin of the left subclavian artery; (7) cardiac hypertrophy and dilatation; (8) acute passive hyperemia of viscera.

Case 3.—A girl weighing 6¼ lb. was delivered at term and breathed spontaneously at birth. Thirty-three hours later respirations became labored, and this difficulty was more marked during feeding.

Physical Examination.—The rectal temperature was 37.6 C. The baby was in respiratory distress, and there were hoarse grunting cries during expiration, but cyanosis was not present. There was dullness over the left lower lobe of the lung and decreased breath sounds over the entire left lung area. The heart sounds were normal, and no murmurs were heard. The liver was palpable 2 fingerbreadths below the costal margin.

Laboratory Data.—The hemoglobin was 17 gm. per 100 cc., and the white blood cell count, 36,400. A roentgenogram of the chest was not remarkable.

Hospital Course.—Moist rales were heard over the left lower chest on the second day, and the child died three days after birth.

Autopsy Findings.—The heart weighed 14 gm. (normal, 17 gm.). The superior vena cava drained into the right atrium, which was dilated. There was a small opening in the foramen ovale. The right ventricular wall was slightly hypertrophied, and the tricuspid valve was normal. There were multiple small defects between the muscular bundles in the interventricular septum. The pulmonary trunk originated from the right ventricle, close to the interventricular septum, and extended obliquely upward and backward and passed posterior and to the left of the hypoplastic ascending aorta. The pulmonary trunk then curved dorsally and downward as the descending aorta. At the point of origin of the right and left pulmonary arteries the intima was roughened and folded. The pulmonary veins bore their usual anatomic relation to the left atrium. The left atrium was small. There was a small dimple at the left atrioventricular junction, representing the atretic mitral orifice. The mitral valve leaflets were poorly developed and fused. The left ventricle was small, and the wall was one-third the thickness of the right.

The ascending aorta measured 1 cm. in length and originated at the base of the left ventricle and to the right of the pulmonary artery. The innominate and left common carotid arteries arose from the aorta and followed their usual distribution. The left subclavian artery arose at the junction of the pulmonary artery and ductus arteriosus and followed its usual courses. The arch of the aorta was absent. There was no communication between the ascending aorta and either the pulmonary trunk or the descending aorta. The circumference of the ascending aorta was approximately half that of the pulmonary artery. Both coronary ostia were in their usual location. There was a biscuspid aortic valve.

Summary of Postmortem Findings: (1) absence of the transverse aortic arch; (2) hypoplasia of ascending aorta; (3) biscuspid aortic valve; (4) atresia of the mitral orifice; (5) patent foramen ovale; (6) interventricular septal defects; (7) passive hyperemia of viscera; (8) atelectasis of upper left lung; (9) an accessory spleen.

Case 4.—A boy weighing 2 lb. ½ oz. was stillborn after seven and one-half months' gestation. The mother was Gravida IV and Para III and had polyhydramnios. There was a history of one miscarriage after four and one-half months' gestation three years previously.

Postmortem Findings.—The heart consisted of two chambers, the walls of which were thin and flabby. It weighed 10 gm. with the great vessels attached. The inferior and superior vena cavae entered the common atrium. The common ventricle was four times larger than the atrium and communicated with it by a narrow orifice which had no definite cusps. A small ascending aorta and a larger pulmonary trunk arose from the base of the ventricle. The ascending aorta gave off two branches, one to each side of the neck. The pulmonary trunk gave off a branch to each lung and then continued to join the descending aorta. The aortic valve was bicuspid, and two coronary ostia arose from the sinuses. The pulmonary valve had three cusps.

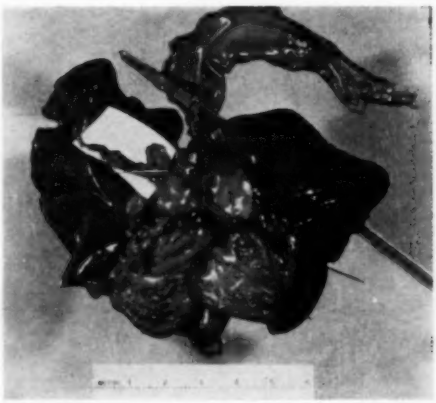

Fig. 3 (Case 4).—The heart is opened so that the right side of the interventricular septum is visible. The high interventricular septal defect is present. The hypoplastic ascending aorta with the terminal branches, the innominate and left common carotid arteries, lie on the white card. The large probe is in the left subclavian artery.

Summary of Postmortem Findings: (1) absence of transverse aortic arch; (2) hypoplasia of the ascending aorta; (3) bicuspid aortic valve; (4) cor biloculare; (5) anencephaly; (6) bilateral harelip and cleft palate; (7) heterotopia spinalis; (8) hypoplasia of left lung; (9) malrotation of intestine; (10) absence of left diaphragm.

Case 5.—This was a full-term stillborn macerated female fetus. The birth weight was 5 lb. 9 oz. The mother was Gravida XIII and Para IX and had had three abortions. The mother's blood was Type A Rh-negative and the father's was Type O Rh-positive. Serologic test for syphilis in the second trimester was negative.

Postmortem Findings.—The heart was enlarged and weighed 25.8 gm. (normal, 17 gm.) with the great vessels attached. There was complete absence of the aortic arch. The aorta measured 1.7 cm. in circumference. It branched into the right and left common carotid arteries. The pulmonary trunk was large, measuring 2.8 cm. in circumference, and occupied three-fourths of the base of the heart. It did not communicate with the ascending aorta but was continuous with the descending thoracic aorta. The pulmonary trunk branched into the right and left pulmonary arteries. Only two pulmonary veins could be identified, and they emptied into the

Fig. 4 (Case 5).—*A*, descending aorta. *S*, origins of the right and left subclavian arteries at junction of the ductus arteriosus and pulmonary artery. *C*, right and left carotid arteries arising from the hypoplastic ascending aorta. *P*, the branching pulmonary arteries.

left atrium. The right and left subclavian arteries arose at the junction of the pulmonary trunk and the descending aorta, the right passing behind the trachea and esophagus.

The foramen ovale was widely patent. The ventricles communicated by a septal defect measuring 1.2×0.7 cm. located in the anterosuperior portion of the septum. The right coronary artery arose from the ascending aorta, and the left coronary artery, from the anterior portion of the pulmonary trunk. The leaflets of the mitral valve were normal. The tricuspid valve was not remarkable. The aortic valve had one normal and two hypoplastic cusps. The pulmonic valve had a small accessory and three normal cusps.

Summary of Postmortem Findings: (1) absence of the aortic arch; (2) hypoplasia of the ascending aorta; (3) hypoplasia of two aortic valve cusps; (4) interventricular septal defect; (5) patent foramen ovale; (6) anomalous origin of the subclavian arteries from descending aorta; (7) accessory cusp on pulmonary valve; (8) cardiac hypertrophy and dilatation; (9) nonpneumatization of lungs; (10) recent hemorrhage of falx cerebri.

Case 6.—A premature white boy, approximately 35 gestatory weeks of age and weighing 1750 gm., was delivered by low forceps and with use of spinal anesthesia.

At birth the child was limp and pallid. Respirations were intermittent and gasping. The heart sounds were of fair quality. There was marked bradycardia. The ears were large and soft, with absence of cartilage, and set low on the head. The liver was palpable 1 ½ fingerbreadths below the costal margin. There were bilateral club feet.

Laboratory Data.—Hemoglobin 16 gm. per 100 cc.; blood Type O Rh-positive; Coombs test negative.

The mother was Gravida III and Para II, 19 years of age, and healthy. Her serology was negative, and the pregnancy was uneventful. She was Rh-negative.

The child died approximately two and one-half hours after birth, in respiratory distress.

Postmortem Findings.—The heart weighed 14 gm. (normal, 15 gm.). There was a defect measuring 0.4 cm. in diameter in the membranous portion of the interventricular septum. The foramen ovale was patent and measured 0.8 cm. in diameter. It was partially covered by a membranous crescentic fold 2 mm. in width. The tricuspid, mitral, and pulmonic valves were normal. The aortic valve was biscuspid, the right part being larger than the left. A single coronary ostium was present above the medial half of the right cusp. The aortic ring was 1.2 cm. in circumference, as compared to the pulmonic circumference of 1.9 cm. The aorta did not override the ventricular septal defect. The ascending portion of the aortic arch was present, but the arch was absent. The ascending aorta terminated by dividing into two innominate arteries.

Fig. 5 (Case 6).—The heart is opened, and the left side of the interventricular septum is exposed. Note the bicuspid aortic valves immediately above the interventricular septal defect. *IVS*, interventricular septum. *A*, ascending aorta. *PA*, pulmonary artery. *IN*, innominate arteries. *RPA*, right pulmonary artery. *LPA*, left pulmonary artery. *DUC ART*, ductus arteriosus. *DA*, descending aorta.

The main pulmonary artery gave off the left and right branches, and just distal to the origin of the left pulmonary artery the ductus arteriosus was present. It was patent and connected the pulmonary artery to the descending thoracic aorta. The ductus measured 0.8 cm. in width and 1 cm. in length. The abdominal aorta was not unusual except that no renal arteries could be identified as arising from it.

The left kidney was absent. The right kidney was immediately adjacent to the right adrenal and was smaller than usual, measuring 1.8×0.7×0.4 cm. Its surface was lobulated, but no corticomedullary demarcation was present, and the renal pelvis was not identified. The distal portion of the right ureter was atretic. The lungs were markedly hyperemic and nonpneumatized.

Summary of Postmortem Findings: (1) absence of transverse aortic arch; (2) hypoplasia of the ascending aorta; (3) bicuspid aortic valve; (4) interventricular septal defect; (5) patent foramen ovale; (6) agenesis of the left kidney and hypoplasia of right kidney; (7) marked hyperemia and nonpneumatization of lungs; (8) prematurity (1750 gm.).

Comment

Anatomic Lesions.—The cardiac and vascular defects in the present 6 cases and in 12 of the cases previously reported in the literature are listed in Table 1. Hypoplasia of the ascending aorta was present in 12 of the 15 cases in which adequate description was given. In two of the three cases in which the ascending aorta was not described as hypoplastic, the circumferences were listed as 17 mm. and 14 mm. Malformations of the cusps of semilunar valves were present in 9 of 15 cases, and of these the aortic valve cusps were involved 8 times. Seven of the aortic valve deformities took the form of bicuspid valves. The foramen ovale was patent in 12 of 17 cases. One of the other cases was a cor biloculare. A ventricular septal defect was present in 16 of 18 listed cases, and again 1 of the 2 remaining was the cor biloculare. The ventricular defect

TABLE 1.—*Cardiovascular Lesions of Collected Cases of Absence of Transverse Aortic Arch* *

Authors	Absent Transverse Aortic Arch	Hypoplasia Ascending Arch	Malformation, Semilunar Valve Cusps	Patent Foramen Ovale	Ventricular Septal Defect	Hypertrophy Ventricle	Anomalous Origin of Carotid & Subclavian Vessels	Mitral Atresia	Transposition Great Vessels	Other Cardiovascular Anomalies
Gaspar	+	+	0	+	+	R-L	L sub arises junct. PA, DA & desc. aorta	0	0	0
Abbott	+	+	+	—	+	—	L sub arises desc. aorta	+	+	Common atrioventricular canal
Evans 1	+	+	—	+	+	R-L	R & L sub arise desc. aorta	0	0	0
2	+	+	—	+	+	—	Small L sub arises desc. aorta	0	0	0
3	+	—	—	+	0	0	0	0	0	0
Hamburger	+	—	—	+	+	L	In L CC & L sub off ascend. aorta	0	+	0
Weisman & Kesten	+	+	+	+		+	L CC & L sub off ascend. aorta to PA	0	0	0
Stewart	+	—	—	—	+	—	L sub, LCC & In off ascend. aorta	0	0	0
Jew & Gross	+	0	0	+	+	R-L	L sub off junct. DA & desc. aorta	0	0	R PA originates from aorta
Kintner	+	0	0	0	+	R	All vessels arise from ascend. aorta	+	0	Persistent L superior vena cava
Barger et al.	+	0	—	—	+	—	L sub off desc. aorta opposite DA	0	0	0
Jew & Gross	+	+	+	—	+	R	R sub off R In; L sub unknown	0	0	—
Present authors 1	+	+	+	+	+	R	L sub off junct. PA & DA; R sub off PA distal to L sub	0	0	R desc. aorta
2	+	+	+	+	+	0	L sub off junct. PA & DA	0	0	—
3	+	+	+	+	+	R	L sub off junct. PA & DA	+	0	0
4	+	+	+	0	0	0	L sub not identified	0	0	Cor biloculare
5	+	+	+	+	+	+	R & L sub off junct. PA & DA	0	0	L coronary art. arises from PA
6	+	+	+	+	+	0	L sub off L In	0	0	0

* + indicates present; 0, absent; —, no data; sub, subclavian artery; PA, pulmonary artery; DA, ductus arteriosus; CC, common carotid; In, innominate.

was frequently seen in the superior membranous portion of the interventricular septum. Cardiac hypertrophy was present in 10 of 12 cases and was usually of the right ventricle. Mitral atresia was present in only 3 of 17 cases, and transposition of the great vessels was noted twice.

In seven cases the ascending aorta gave origin to the innominate and left common carotid arteries, and the left subclavian arose just distal to the junction of the descending aorta, the ductus arteriosus, and the main pulmonary trunk. In four cases the innominate, left common carotid, and left subclavian all arose from the ascending aorta. In three cases both the right and the left subclavian arteries rose from the descending aorta and pulmonary artery, while the ascending aorta gave off only the right and left common carotids. In one case the left common carotid and left subclavian arteries arose from the descending aorta and the pulmonary artery. In three cases the left subclavian origin is not described. Abbott [1] has described two types of absence of the transverse aortic arch. The first, and commoner, type is that in which the subclavian and carotid vessels arise from the ascending aorta. This type she regarded as a severe form of infantile coarctation of the aorta in which the stenosed part is completely absent. In the rarer type, the aortic arch is absent just after the origin of the left carotid artery and before the origin of the left subclavian artery, which is given off from the descending aorta. However, at least two other types exist. These are (*a*) origin of both right and left subclavian vessels from descending aorta and (*b*) origin of left common carotid and left subclavian arteries from the descending aorta.

Hypoplasia of the ascending aorta; malformation of the semilunar valve cusps, particularly the aortic cusps, and a defect in the membranous portion of the ventricular septum are frequently associated with absence of the transverse aortic arch. That this association is not merely coincidence is indicated by consideration of the embryologic development of these structures.

Embryologic Considerations.—At about the third week of embryonic life, the human embryo begins to form six pairs of aortic arches connecting ventral and dorsal aortae. The first two pairs degenerate before the posterior arches have been established, but their disappearance is neither abrupt nor complete. The third arches develop into the internal carotids. The fourth aortic arch has a different fate on opposite sides of the body. On the left it persists as the arch of the adult aorta; on the right it forms the proximal part of the subclavian artery. The fifth arch on each side has a transitory existence. The sixth aortic arch changes its original relationships more than the others. At an early stage of development branches extend from its right and left portions toward the lungs. After these pulmonary vessels have been established, the right side of the sixth aortic arch loses communication with the dorsal aortic root and disappears. On the left, however, the sixth arch retains its communication with the dorsal aortic root and is the ductus arteriosus. As the paired aortic arch system is evolving and involuting, the primitive cardiac tube is bending to form the atria and ventricles. The most cephalic part of the cardiac tube persists as the truncus arteriosus, connecting the ventricle with the ventral aortic roots. A bulging portion of the truncus arteriosus is the site of division into separate pulmonary and aortic roots. At this site, even at an early stage (6 mm. embryo), there is a primitive cushion of endocardial tissue extending as two longitudinal ridges in a clockwise spiral path from the cephalic end of the truncus arteriosus through the conus to the ventricle.[2] This ridge system can be separated into three sections: 1. The region of the ventral aortic roots at the level where the fourth and sixth left aortic arches are to be separated. This ridge, when completed, forms the septum aortopulmonale between the origins of the ascending aorta and the main pulmonary artery. 2. The level where

the aortic and pulmonary valves are formed. 3. The ventricular side of the semilunar valves. In the latter region the conus portion of the truncus ridges meet with the interventricular septum and certain local enlargements of the atrioventricular canal cushion to complete the partitioning of the ventricles in the membranous portion.

Correlated anatomic defects exist in all three portions of the developing truncus ridges in the majority of cases with absent transverse aortic arch. The frequent occurrence of hypoplasia of the ascending aorta, malformation of the semilunar valve cusps, and a defect in the membranous portion of the interventricular septum appear to be reasonable evidence of a defect in the embryologic development of the truncus ridges in cases with absent transverse aortic arch.

The adult aortic arch itself is derived embryologically from several structures, including (*a*) the aortic trunk, which develops into the proximal end of the arch from the valves to the innominate artery; (*b*) the left half of the aortic sac, which develops into the portion of the arch between the innominate and left common carotid arteries, and (*c*) the left fourth aortic arch together with the paired aortae between it and the developing pulmonary (sixth) arch, which is the ancestral site of the portion of the arch between the left common carotid artery and the ductus arteriosus.

An analysis of the origins of carotid and subclavian vessels in our cases indicates that it is the latter portion of the aortic arch, that derived from the fourth left aortic arch and the paired aortae between it and the sixth arch, which is deficient. However, in at least one other case, that in which the left common carotid and left subclavian arteries arose from the descending aorta and pulmonary artery, there may be additional deficiencies in the formation of the aortic arch. The anomalous origin of the right subclavian with the left subclavian from the descending aorta need not involve a further defect in the aortic-arch-system components.

The absence of the fourth left aortic arch is probably the result of an abnormal involution of this structure rather than a complete lack of development embryologically. Ample evidence is present from embryologic studies of formation and subsequent involution of other portions of the embryonic aortic arch system, including the first, second, and fifth arches. In his excellent discussion of the transformation of the aortic arch system, Congdon [3] repeatedly stresses the influence of the dynamic environmental processes on the development of the vascular system and states, "The chief cause of the disappearance of the first and second arches is probably to be found in the shift of the blood stream to the more caudal arches—which accompanies the caudal movement of the aortic sac."

With this background of the influence of dynamic development of the arch system in the embryo it seems reasonable to attempt to associate the malformations of the truncus ridges and the absent fourth left aortic arch. The defective partitioning of the septum aortopulmonale with its attendant formation of a hypoplastic ascending aorta as well as the defective formation of the aortic valve cusps may well act to shunt the main stream of blood flow in the embryo from its normal course in the fourth left arch to the enlarged sixth arch. This relative disuse of the fourth arch possibly could be instrumental in causing an involutionary change, with ultimate hypoplasia.

Clinical Analysis.—In Table 2 are summarized 12 cases which we have personally reviewed and the 6 cases reported here. Of 17 cases in which the sex of the infant was noted, 9 were boys and 8, girls. The age of survival varied from stillbirth to 180 days, and the average survival time was 15.2 days, but if the single 180-day value is omitted, the mean survival age is 4.9 days. There appeared to be no consistent prenatal factor associated with the appearance of this anomaly. The majority of the mothers were multigravida. There was no history of infectious disease or blood incompatibilites in

TABLE 2.—*Clinical Data of Collected Cases of Absence of Transverse Aortic Arch*

Author	Sex	Age, Days	Cyanosis	Mongolism	Cardiac Murmurs	Respiratory Distress	Cardiac Enlargement	Other Lesions & Congenital Defects
Gaspar	F	180	+	0	+	−	+	Bilat. otitis media
Abbott	−	15 hr.	+	+	−	−	−	−
Evans 1	M	3	−	−	−	−	−	−
2	F	11	−	−	−	−	−	−
3	M	2	−	−	−	−	−	−
Hamburger	M	30	+	0	+	+	+	−
Weisman & Kesten	M	3	+	0	0	−	−	Passive hyperemia of lungs and viscera
Stewart	F	2	+	0	0	+	0	Atelectasis, left lung
Jew & Gross	M	3	+	0	+	+	−	−
Kintner	F	14.5 hr.	+	0	0	0	+	Accessory spleen, double cervix & vagina
Barger et al.	F	8	+	0	−	+	+	Marked pulmonary edema; mesenteric attachment of right large bowel
Jew & Gross	F	54 hr.	+	0	0	+	−	−
Present 1 authors	M	8	0	0	0	+	0	Cleft palate
2	M	3	+	0	0	+	0	−
3	F	3	0	0	0	+	0	Pneumonia, hepatomegaly
4	M	Stillborn	−	−	0	−	−	Anencephaly, cleft palate, 3 eyes
5	F	Stillborn	−	0	−	−	−	−
6	M	Premature 2½ hr.	0	0	0	+	0	Agenesis of L kidney; hypoplasia of R kidney

10 of 13 cases in which the data were given. However, there was an Rh incompatibility of the blood of the parents in Case 5.

Cyanosis was noted in 10 of 13 cases in which the data were presented. In 6 of the 10 cases it was noted that the cyanosis came on at varying times after birth, from after the first feeding to the seventh day of life. Cyanosis of the lower portion of the body is said to be characteristic of this malformation, while the upper trunk, head and neck, and some part of the upper extremities are of normal color,[4] but this was observed in only one of our cases (Case 5). In Hamburger's[5] case with transposition of the great vessels, in addition to the absent transverse aortic arch, the cyanosis was more intense above the umbilicus. The intermittency or inconstancy of the cyanosis is probably related to associated cardiac septal defects on malrotation of the trunks of the great vessels which act to shunt some oxygenated blood into the descending aorta and thus mask this sign. It is for this reason that what has been considered the classical manifestation of absence of the transverse aortic arch is not usually observed.

Mongolism was noted once in 15 cases, and this case had an associated common atrioventricular canal.[1] Cardiac murmurs were heard in 3 of 10 cases in which data were presented. Cardiac enlargement was noted in four of eight cited instances, and it is of interest that three of the four cases were among the oldest surviving. Respiratory distress was a common clinical feature, occurring in eight of nine cases in which data were presented. It usually appeared shortly before death. This, plus the frequent postmortem finding of severe hyperemia of the lungs, indicates that terminal cardiac failure probably is a constant feature.

The paucity of definitive clinical signs makes the possibility of antemortem diagnosis unlikely. Cardiac murmurs were either absent or not distinctive enough to be of diagnostic help. This diagnosis may be specifically suggested ante mortem, by the presence of cyanosis restricted to the lower extremities, lower trunk, and possibly the left and right arms, while the head and neck are normal in color. The frequent occurrence of associated septal defects is probably responsible for the fact that this sign is not seen with greater frequency and detracts from its value as a diagnostic aid.

Summary

Six cases of absent transverse aortic arch are presented, bringing the total reported cases to twenty-seven. Infants afflicted with this anomaly usually do not survive longer than 15 days, and the majority survive less

than 5 days. Cyanosis is frequently present and usually appears a few days following birth. Cyanosis of the lower half of the body only, which is said to be diagnostic of absent transverse aortic arch, was seen in only one of the present cases. Respiratory distress was the commonest symptom and usually appeared several hours before death.

Absence of the transverse aortic arch is frequently associated with hypoplasia of the ascending aorta, malformation of the aortic valve, and a defect in the membranous portion of the interventricular septum. This suggests that faulty partition of the truncus arteriosus may be causally related to the abnormal involution of the fourth left aortic arch.

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REFERENCES

1. Abbott, M. E.: Atlas of Congenital Cardiac Disease, New York, The American Heart Association, 1936.

2. Kramer, T. C.: The Partitioning of the Truncus and Conus and the Formation of the Membranous Portion of the Interventricular Septum in the Human Heart, Am. J. Anat. 71:343, 1942.

3. Congdon, E. D.: Transformations of the Aortic-Arch System During the Development of the Human Embryo, Contrib. Embryol. 14:47, 1922.

4. Taussig, H. B.: Congenital Malformations of the Heart, New York, The Commonwealth Fund, 1947.

5. Hamburger, L. P.: Congenital Cardiac Malformation with Complete Interruption of Isthmus, Aorta and Transposition of Great Arteries, Bull. John Hopkins Hosp. 61:421, 1937.

6. Gaspar, I.: Two of the Rare Congenital Anomalies of the Heart, Am. J. Path. 5:285, 1929.

7. Evans, W.: Congenital Stenosis (Coarctation), Atresia and Interruption of Aortic Arch: Study of 28 Cases, Quart. J. Med. 2:1, 1933.

8. Weisman, D., and Kesten, H. D.: Absence of the Transverse Aortic Arch with Defects of Cardiac Septums: Report of a Case Simulating Acute Abdominal Disease in a Newborn Infant, Am. J. Dis. Child. 76:326, 1948.

9. Stewart, M.: Congenital Interruption of Aortic Arch, Arch. Dis. Childhood 23:63, 1948.

10. Jew, E. W., Jr., and Gross, D.: Aortic Origin of the Right Pulmonary Artery and Absence of the Transverse Aortic Arch Associated with Patency of Interventricular Septum and Ductus Arteriosus, A. M. A. Arch. Path. 53:191, 1952.

11. Kintner, E. P.: Congenital Malformation of the Heart: Interruption of Aortic Arch, Mitral Valve Orifice Atresia, and Persistent Left Superior Vena Cava, Lab. Invest. 2:388, 1953.

12. Barger, J. D.; Creasman, R. W., and Edwards, J. E.: Bilateral Ductus Arteriosus Associated with Interruption of the Aortic Arch, Am. J. Clin. Path. 24:441, 1954.

13. Jew, E. W., Jr., and Gross, P.: Absence of Transverse Arch of Aorta Associated with Patency of the Interventricular Septum and the Ductus Arteriosus, Lab. Invest. 4:304, 1955.

Atherostatic and Lipophage-Stimulating Effects of Manuronate

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Introduction

It has been shown previously that sulfated alginic acid (SAA), a nonanticoagulant sulfated polymannuronide, given in the presence of a high cholesterol intake, will prevent the development [1] and arrest the progress [2] of preestablished cholesterol atherosclerosis while suppressing lipemia and stimulating lipid phagocytosis in reticuloendothelial tissues and kidney (i. e., provoking a foam-cell reaction).

The present study shows that Manuronate (Wyeth), an anticoagulant sulfated polymannuronide, has similar atherostatic, antilipemic, and lipophage-stimulating properties. An experimental analysis is made of factors controlling lipid phagocytosis, and it is demonstrated that, under proper conditions, this compound can exert a marked and sustained antilipemic effect without provoking a foam-cell reaction or other undesirable side-effects.

Materials and Methods

A total of 199 rabbits was used in four experiments. In each experiment, the groups under comparison were balanced with regard to litter, sex, and initial body weight and they were match-fed daily to the minimum intake.

EXPERIMENT 1.—This experiment was designed to find out whether high dosages of Manuronate, like SAA, given some time after the development of atherosclerosis and in the presence of continued high cholesterol intake, would affect the preestablished lipemia and atherosclerosis and whether it would elicit any cellular lipophagia (foam-cell reaction) under those circumstances. It was also designed to find out whether Manuronate would produce any foam cells in the absence of a high cholesterol intake.

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A lot of 70 rabbits were given a 1%-cholesterol diet for two months. At the end of this period, the 48 animals that developed comparable serum cholesterol and turbidity levels were divided into four groups of 12 animals each, the remaining 22 rabbits being discarded. The rabbits in Group I were killed immediately, whereas those in Groups II, III, and IV were kept alive for another (third) month before killing. During this third month, the rabbits in Group II continued the cholesterol diet, those in Group III continued the cholesterol diet and received daily subcutaneous Manuronate injections (10 mg. per kilogram), and those in Group IV switched to a normal (cholesterol-free) diet.

Simultaneously, in order to study the effects of Manuronate on macrophages in the absence of cholesterol feeding, the following two additional eight-rabbit groups were tested. The rabbits in Group V were given a normal diet throughout the duration of the experiment, and those in Group VI were similarly given a normal diet throughout the duration of the experiment, but they also received daily subcutaneous Manuronate injections (10 mg. per kilogram) during the third month.

End-points were periodic serum turbidity, cholesterol, phospholipid, and total lipid determinations; aortic atherosclerosis evaluation (plaque area and thickness); determination of splenic mass and cholesterol content, and histological examination of spleen and kidney.

Serum turbidity and total cholesterol were determined as previously [3]; serum phospholipid was calculated by determining lipid phosphorus with a modified method of Youngburg and Youngburg [4] and multiplying the value so obtained by 25; serum total lipids were estimated by the method of Swahn.[4]

The total aortic atheroma area was measured planimetrically as described previously.[2] The maximal thickness of the largest plaque in each aortic arch was measured micrometrically in a Sudan-hematoxylin-stained, formalin-prefixed frozen section made from a minute sample through the center of the highest lesion in every arch; it represented the greatest distance in microns from the base (first intact elastic lamella) to the surface of the atheroma sampled. Splenic cholesterol was

determined by the method of Sperry and Webb.[5] The histological studies were made in hematoylin-and-eosin-stained paraffin sections and in sudan-and-hematoxylin-stained frozen sections from every spleen (midportion) and kidney (whole organ).

EXPERIMENT 2.—This experiment was designed to study the effects of cholesterol intake, pre-established lipemia, and drug-dosage levels on the Manuronate-induced lipophagic reaction as well as to determine the reversibility of this reaction after withdrawal of both the drug and the cholesterol alimentation.

A lot of 60 rabbits were given a cholesterol regimen for seven weeks, half of the animals being given a high- (1%) and the other half a low- (0.5%) cholesterol diet. At the end of this initial seven-week cholesterol-feeding period, 22 animals with the high cholesterol intake that achieved comparably high serum-turbidity and cholesterol values were selected and subjected to daily high-dosage Manuronate injections (10 mg. per kilogram, s. c.), while continuing their high-cholesterol diet, for another (third) month. At the end of this third month, the rabbits given high-cholesterol diets and high-dosage Manuronate injections were divided into two groups, Group I (10 rabbits) being killed immediately, whereas in Group II (12 rabbits) Manuronate was discontinued and they were switched to a normal (cholesterol-free) diet for another two months before killing.

Similarly, 20 animals given the low-cholesterol intake that achieved comparably low serum-turbidity and cholesterol values at the end of the initial two-month cholesterol feeding period were selected and divided into two groups (Groups III and IV) of 10 rabbits each. Both groups were subjected to daily Manuronate injections while continuing their low cholesterol intake for another (third) month before killing, but one (Group III) received a high (10 mg. per kilogram, s. c.), whereas the other (Group IV) received a low (2.5 mg. per kilogram, s. c.) dosage level of this drug.

End-points of this experiment were the same as those of the preceding one, with the exception of serum phospholipids, total lipids, and atherosclerosis, which were not studied.

EXPERIMENT 3.—This experiment was designed to restudy the effects of drug dosage and cholesterol-intake level under conditions of exceedingly high preestablished lipemia and prolonged treatment.

A lot of 45 rabbits were given a high- (1%) cholesterol diet for 10 weeks. At the end of this period, the 24 animals that achieved comparably excessive serum turbidity and cholesterol values were selected and divided in three groups of 8 rabbits each. All three groups were subsequently subjected to daily Manuronate injections while continuing on various cholesterol intakes for another two months before killing, as follows:

Group I, high cholesterol (1%) intake and high Manuronate (10 mg. per kilogram) dosage; Group II, low cholesterol (0.5%) intake and high Manuronate (10 mg. per kilogram) dosage; Group III, high cholesterol (1%) intake and low Manuronate (2.5 mg. per kilogram) dosage.

TABLE 1 (Experiment 1).—*Blood Lipids (Means ±e)*

Group	Day 0 *	Day 58 *	Day 64 *	Day 70 *	Day 83 *
A. Serum Turbidity, Klett Units					
I. Early cholesterol controls	5±0.7	52±6.8	Killed		
II. Late cholesterol controls	5±0.7	54±6.8		65±6.4	49±7.2
III. Manuronate plus cholesterol	5±0.7	51±6.7	Injections started	11±1.2	8±1.4
IV. Cholesterol withdrawal	5±0.7	53±6.1	Cholesterol withdrawn	14±2.1	12±1.3
B. Serum Total Cholesterol, Mg./100 Ml. Serum					
I. Early cholesterol controls	70±4.5	1048±109	Killed		
II. Late cholesterol controls	70±4.5	1066±90		--	1224±116
III. Manuronate plus cholesterol	70±4.5	1007±103	Injections started	--	353±47
IV. Cholesterol withdrawal	70±4.5	1023±85	Cholesterol withdrawn	--	418±72
C. Serum Phospholipids, Mg./100 Ml. Serum					
I. Early cholesterol controls	109±11	464±31	Killed		
II. Late cholesterol controls	109±11	496±36		--	567±57
III. Manuronate plus cholesterol	109±11	438±40	Injections started	--	219±17
IV. Cholesterol withdrawal	109±11	465±40	Cholesterol withdrawn	--	295±21
D. Serum Cholesterol:Phospholipid Ratio					
I. Early cholesterol controls	0.82±0.16	2.26±0.18	Killed		
II. Late cholesterol controls	0.82±0.16	2.15±0.13		--	2.20±0.20
III. Manuronate plus cholesterol	0.82±0.16	2.29±0.13	Injections started	--	1.55±0.12
IV. Cholesterol withdrawal	0.82±0.16	2.21±0.10	Cholesterol withdrawn	--	1.61±0.13
E. Serum Total Lipids, Mg./100 Ml. Serum (Referred to a Corn-Oil Standard)					
I. Early cholesterol controls	367±72	2985±434	Killed		
II. Late cholesterol controls	367±72	2628±257		--	3501±243
III. Manuronate plus cholesterol	367±72	2592±340	Injections started	--	956±123
IV. Cholesterol withdrawal	367±72	3415±423	Cholesterol withdrawn	--	--

* Days are numbered with reference to the commencement of cholesterol feeding.

End-points of this experiment were the same as those of Experiment 2, with the exception of splenic cholesterol, which was not determined.

EXPERIMENT 4.—This experiment was expected to show whether the intermittent administration of low dosages of Manuronate would have any anti-lipemic or lipophagia-inducing effects.

A lot of 24 rabbits were given a low- (0.5%) cholesterol diet for two months. At the end of this period, 16 rabbits that achieved comparable serum turbidity values were selected and divided into two groups of 8 animals each. Both groups continued on the 0.5%-cholesterol diet for another two months before killing, but Group I received no treatment, whereas Group II received low dosages of Manuronate (2.5 mg. per kilogram) three times weekly, throughout the latter two months.

End-points of this experiment were the same as those of Experiment 3.

Results

EXPERIMENT 1 (Tables 1-3).—As can be seen in Table 1, cholesterol feeding for two months (Group I) raised the serum-turbidity, cholesterol, phospholipid, cholesterol-phospholipid (C:P) ratio, and total-lipid levels to high values. An additional (third) month of cholesterol feeding (Group II) maintained these values at the same high levels or tended to increase them even further. Manuronate, given concurrently with cholesterol feeding during the third month (Group III), wiped out the serum lactescence and it depressed the serum cholesterol, phospholipid, C:P ratio, and total lipid levels by 65%, 50%, 32%, and 63%, respectively. This normaling effect of Manuronate on the preestablished lipemia (which was exerted despite the continued high cholesterol intake) was comparable to or even greater than the effect of dietary cholesterol withdrawal (Group IV).

TABLE 2 (Experiment 1).—*Aorta*

Group	Mean Aortic Area Occupied by Plaques ± σ, %	Mean Maximum Thickness of Largest Plaque in Aortic Arch ± σ, μ
I. Early cholesterol controls	11.0±4.9	118±32
II. Late cholesterol controls	22.7±5.0	243±30
III. Manuronate plus cholesterol	12.6±4.3	161±25
IV. Cholesterol withdrawal	14.4±4.4	214±33

Correspondingly, Table 2 shows that continued cholesterol feeding during the third month doubled the area and the thickness of the lesions that had been produced during the preceding two months, whereas Manuronate treatment, given together with cholesterol feeding, arrested the growth of both the total preestablished atheroma area and the thickness of the upper aortic plaques. It is of interest that despite dietary cholesterol withdrawal the arch lesions of Group IV tended to grow in thickness during the third month ($P=0.05$).

It should be further noted that the over-all incidence of medial calcification in this experiment was low and of a slight degree wherever it occurred, without significant predilection for any group.

Table 3 shows that Manuronate, given in the presence of a high cholesterol intake, caused a considerable and significant increment of the splenic mass, cholesterol content, and foam-cell reaction and it provoked a marked renal foam-cell reaction, while arresting body growth. Given at the same high-dosage level in the absence of dietary cholesterol, however, the drug failed to increase the splenic weight and cholesterol content or to produce foam cells in spleen

TABLE 3 (Experiment 1).—*Spleen and Kidney*

Group	Mean Splenic Wt. ± σ (Mg./Kg.)	Mean Splenic Cholesterol ± σ Mg./Spleen	Splenic Foam-Cell Reaction		Renal Foam-Cell Reaction		Mean Body Wt. Change During Third Month, % of Preinjection Value
			Incidence, %	Mean Degree	Incidence, %	Mean Degree	
I. Early cholesterol controls	--	--	18	0.7	0	--	--
II. Late cholesterol controls	550±65	57±5	54	2.3	0	--	+3.6
III. Manuronate plus cholesterol	1420±166	245±44	92	3.6	80	1.3	+0.9
IV. Cholesterol withdrawal	412±63	18±5	9	1.5	0	--	+5.4
V. Absolute controls	389±35	5.8±0.9	0	--	0	--	+12.0
VI. Manuronate alone	422±38	5.9±0.4	0	--	0	--	+10.7

TABLE 4 (Experiment 2).—*Blood Lipids*

Group	Day 0	Day 45	Day 40	Day 63	Day 77
		A. Serum Turbidity, Klett Units			
I. High Manuronate-high cholesterol	4±2.4	64±9.6	Injections started	11±2.4	15±3.1
II. High Manuronate-high cholesterol, followed by withdrawal of both	4±2.4	63±5.5	Injections started	8±1.9	14±3.3
III. High Manuronate-low cholesterol	4±2.4	22±1.3	Injections started	4±0.7	3±0.9
IV. Low Manuronate-low cholesterol	4±2.4	26±5.3	Injections started	4±1.0	3±0.7
		B. Serum Cholesterol, Mg./100 Ml. Serum			
I. High Manuronate-high cholesterol	62±7	945±102	Injections started	426±53	531±68
II. High Manuronate-high cholesterol, followed by withdrawal of both	62±7	1095±89	Injections started	466±62	512±56
III. High Manuronate-low cholesterol	62±7	568±134	Injections started	244±43	--
IV. Low Manuronate-low cholesterol	62±7	558±60	Injections started	265±27	207±31

and kidney and it permitted normal body growth (Group VI).

It should be underlined that the lipid nature of the substance accumulating in the foam cells was proven histologically by the excellent correlation between vacuoles in paraffin sections and sudanophilic material in frozen sections from every animal in every group.

Intercurrent accidents and infections accounted for the loss of one rabbit in each of Groups I, II, and IV.

EXPERIMENT 2 (Tables 4 and 5).—The data of Table 4 demonstrate that high dosages of Manuronate attacked a high lipemia (Groups I and II) as effectively as a moderate lipemia (Group III) and that low dosages of Manuronate were as effective as high dosages in attacking the latter (Group IV).

In Table 5, comparison of Groups I and II shows that two months' withdrawal of both Manuronate and cholesterol feeding led to a return of the enlarged spleen to normal size and to a disappearance of its foam cells and accumulated cholesterol.

In the same Table, comparison of Groups I and III shows that at a constant (high) Manuronate dosage lowering of the cholesterol intake greatly depressed the splenic cholesterol uptake and altogether eliminated the splenic foam-cell reaction. Finally, comparison of Groups III and IV demonstrates that at a constant (low) cholesterol intake lowering of the Manuronate dosage prevents splenic enlargement and significantly depresses splenic cholesterol uptake. Growth was retarded at the high dosage level, but it proceeded normally at the low dosage level of the drug. For clinical purposes, the most important result of this experiment was that at the 2.5 mg. per kilogram dosage level Manuronate exerted a prompt and sustained depressor effect on a hypercholesterolemia of the human order of magnitude (around 500 mg. per 100 cc.) without provoking any splenic or renal foam-cell reaction or interfering with normal growth.

There was one death due to intercurrent infection in Group II.

EXPERIMENT 3 (Tables 6 and 7).—This experiment showed that low dosages of Manuronate were almost as effective as high dosages in attacking an exceedingly high preestablished hypercholesterolemia and in maintaining its antilipemic effect over a longer cholesterol-feeding period than that tested in the preceding experiment. Further-

TABLE 5 (Experiment 2).—*Spleen and Kidney*

Group	Mean Splenic Wt. ± σ, Mg./Kg.	Mean Splenic Cholesterol ± σ, Mg./Spleen	Splenic Foam-Cell Reaction		Renal Foam-Cell Reaction		Mean Body Wt. Change During Injection Period, % of Pretreatment Value
			Incidence, %	Mean Degree	Incidence	Mean Degree	
I. High Manuronate-high cholesterol	1193±147	76.2±23	40	1.9	0	--	+2.6
II. High Manuronate-high cholesterol, followed by withdrawal of both	508±81	1.0±0.1	0	--	0	--	+2.9
III. High Manuronate-low cholesterol	874±104	18.6±2.7	0	--	0	--	+2.7
IV. Low Manuronate-low cholesterol	493±45	11.1±2.4	0	--	0	--	+9.6

TABLE 6 (Experiment 3).—*Blood Lipids*

Group	Day 0	Day 63	Day 71	Day 91	Day 119
		A. Serum Turbidity, Klett Units			
I. High Manuronate-high cholesterol	5±1.2	81±11	Injections started	17±2.3	24±5.8
II. High Manuronate-low cholesterol (during injections)	5±1.2	83±11	Injections started	15±2.8	20±3.1
III. Low Manuronate-high cholesterol	5±1.2	82±12	Injections started	28±5.4	26±6.7
		B. Serum Cholesterol, Mg./100 Ml. Serum			
I. High Manuronate-high cholesterol	42±6	2166±46	Injections started	..	842±71
II. High Manuronate-low cholesterol (during injections)	42±6	1736±79	Injections started	..	724±51
III. Low Manuronate-high cholesterol	42±6	1701±111	Injections started	..	993±106

more, low dosages of Manuronate provoked no renal foam-cell reaction or growth arrest, and they produced only a minimal splenic reaction. By contrast, high dosages of Manuronate elicited, under the conditions of this experiment, marked renal as well as splenic foam-cell reactions, these effects being moderately more prominent on the high than on the low cholesterol-intake level. Microscopic evidence of mild basophilic media necrosis was present in a few arcuate and interlobular renal arteries of some animals in Group I (high Manuronate and high cholesterol), but it was not encountered in the other two groups.

EXPERIMENT 4 (Tables 8 and 9).—The data of this experiment indicate that even intermittent treatment with low dosages of Manuronate has a significant—though slight—depressor effect on a moderate lipemia of long standing, without producing any splenic or renal foam-cell reaction or growth arrest.

Comment

Since Manuronate simulates the biological properties of SAA, it is probable that most sulfated polysaccharides with a mannuronic acid repeat unit can suppress exogenous lipemias of long standing and can arrest the progress of preestablished atherosclerosis. The remarkable antilipemic potency of these agents is illustrated by the fact that Manuronate, given in the presence of continued high cholesterol intake, depressed the elevated blood lipids as effectively as cholesterol withdrawal. The commercial availability and relative inexpensiveness of Manuronate should make it a convenient representative of this class of compounds for further experimental and clinical studies.

As far as the foam-cell reaction (f. c. r.) is concerned, the data of this investigation proved conclusively (*a*) that the material filling the foam cells of Manuronate-treated lipemic animals is indeed lipid and (*b*) that it is exogenous fat, removed from the blood by macrophages, not endogenous material produced by local or systemic lipogenesis.

Since it can be easily computed that the cholesterol accumulating under the influence of Manuronate in the spleen represented only a fraction of that cleared out of the blood stream or absorbed from the intestine, it seems that cholesterol accumulated in foam cells merely because it was "pushed" into the cells faster than they could eliminate it. All observed facts are compatible with this hypothesis. Thus, the f. c. r. was maximal when both the cholesterol load pre-

TABLE 7 (Experiment 3).—*Spleen and Kidney*

Group	Mean Splenic Wt. ± *e*, Mg./Kg.	Splenic Foam-Cell Reaction		Mean Kidney Wt. ± *e*, Mg./Kg.	Renal Foam-Cell Reaction		Mean Body Wt. Change During Treatment, % of Pretreatment Value
		Incidence, %	Mean Degree		Incidence, %	Mean Degree	
I. High Manuronate-high cholesterol	2429±262	88	3.6	4262±272	88	1.7	—0.8
II. High Manuronate-low cholesterol (during injections)	1917±418	88	2.8	3956±158	50	1.4	—1.1
III. Low Manuronate-high cholesterol	932±176	63	2.0	3144±175	0	0	+3.5

TABLE 8 (Experiment 4).—*Blood Lipids*

Group	Day 0	Day 60	Day 61	Day 70	Day 120
		A. Serum Turbidity, Klett Units			
I. Cholesterol	4±1.8	27±2.8	--	26±4.0	31±4.5
II. Cholesterol plus low Manuronate 3 times weekly	4±1.8	27±2.0	Injections started	17±1.7	21±2.0
		B. Serum Cholesterol, Mg./100 Ml. Serum			
I. Cholesterol	58±8	853±120	--	--	849±86
II. Cholesterol plus low Manuronate 3 times weekly	58±8	981±35	Injections started	--	829±41

sented to the cells and the "pushing" force were high (high cholesterol intake and high Manuronate dosage combination); it was severely reduced in intensity when either the cholesterol load alone or the Manuronate dosage alone was reduced, and it disappeared when both factors were simultaneously reduced—without loss of the antilipemic action of the drug. Since under the latter conditions cholesterol might be eliminated as fast as it entered the macrophages, the absence of an f. c. r. need not, of course, exclude the participation of reticuloendothelial elements in the task of lipemia clearing.

It appears that the renal f. c. r. is more difficult to elicit than the splenic one and that, in addition to drug dosage and lipid load, it is partly determined by the preestablished lipemia level. Thus, the renal f. c. r. never appeared in any of three groups testing low dosages of Manuronate (Groups IV, III, and II in Experiments 2, 3, and 4, respectively); among the high-dosage studies it appeared in all three groups in which the preestablished blood-cholesterol level was 1500 mg. per 100 cc. or more (Groups I and II of Experiment 3 and Group IV of a previous investigation [2]), but it occurred variably at the 1000 mg. per 100 cc. level (Group III of Experiment 1 and Group I of Experiment 2). The finding of some media necrosis in renal arteries of the animals with the most prominent renal f. c. r. (Group I of Experiment 3) would be in line with the observed association between aortic media lesions and kidney damage.[6]

It is of considerable interest that the f. c. r. proved completely reversible. Unlike the cholesterol trapped in aortic atheromata,[6] that deposited in the spleen under the influence of high dosages of Manuronate disappeared entirely (along with the foam cells) within two months of the withdrawal of both the drug and dietary cholesterol.

The results of this study provide an experimental justification for clinical trials of low dosages of Manuronate (2.5 mg. per kilogram s. c.) in selected cases of lipemia and hypercholesterolemia. Since human blood cholesterol values rarely reach the dramatically high levels produced by protracted high cholesterol intake in rabbits, it might be expected that within the ordinary range of clinical hypercholesterolemia (300 to 600 mg. per 100 cc.), low dosages of Manuronate would attack the abnormal blood lipid pattern without eliciting any f. c. r. or other untoward side-effect.

Summary

Injections of Manuronate (Wyeth), an anticoagulant sulfated polymannuronide,

TABLE 9 (Experiment 4).—*Spleen and Kidney*

Group	Mean Splenic Wt. ± σ, Mg./Kg.	Splenic Foam-Cell Reaction		Mean Kidney Wt. ± σ, Mg./Kg.	Renal Foam-Cell Reaction		Mean Body Wt. Change During Treatment, % of Pretreatment Value
		Incidence, %	Mean Degree		Incidence, %	Mean Degree	
I. Cholesterol	560±65	12	0.8	3061±95	0	0	+9.4
II. Cholesterol plus low Manuronate 3 times weekly	634±66	25	0.5	3224±168	0	0	+7.2

started in cholesterol-fed rabbits some time after the establishment of severe lipemia and carried on in the presence of a continued high cholesterol intake, wiped out the serum lactescence, markedly reduced the elevated serum cholesterol, cholesterol-phospholipid (C:P) ratio, and total lipid values, and arrested the progress of aortic atherosclerosis. Simultaneously this compound caused the accumulation of cholesterol and lipid-laden foam cells in the spleen, and it caused the appearance of lipophages in renal glomeruli (foam-cell reaction).

In a further series of experiments, various dosage levels of Manuronate were given for several months to groups of animals with various degrees of preestablished lipemia, in the presence of various levels of continued high cholesterol intake.

It was found that, when other factors were kept constant, the ensuing foam-cell reaction was proportional to (*a*) the dosage of Manuronate, (*b*) the lipid intake during treatment, and (*c*) the level of preestablished lipemia. Given at a low dosage to animals with an initial serum cholesterol of 300 to 700 mg. per 100 cc., Manuronate exerted a marked and sustained antilipemic effect without eliciting any foam-cell reaction or other untoward phenomenon. The marked foam-cell reaction which was produced with the high drug dosage and an initial cholesterol of 1700 to 2300 mg. per 100 cc. proved completely reversible following withdrawal of both the drug and cholesterol feeding.

The significance of these findings in relation to clinical trials of sulfated polymannuronides is discussed.

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REFERENCES

1. Gutmann, N., and Constantinides, P.: A. M. A. Arch. Path. 59:717, 1955.

2. Constantinides, P.; Saunders, P., and Wood, A.: A. M. A. Arch. Path. 62:369, 1956.

3. Hawk, P. B.; Oser, B. L., and Summerson, W. H.: Practical Physiological Chemistry, Ed. 13, New York, The Blakiston Company (division of McGraw-Hill Book Company, Inc.), 1954, p. 589.

4. Swahn, B.: Scandinav. J. Clin. & Lab. Invest. (Supp. 9) 5:1, 1953.

5. Sperry, W. M., and Webb, M.: J. Biol. Chem. 187:97, 1950.

6. Constantinides, P., and Saunders, P.: A. M. A. Arch. Path. 65:360, 1958.

Rupture of a Tuberculous Aneurysm of the Aorta

Review of the Literature and Report of a Case

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The purpose of this paper is to present a case of fatal hemorrhage resulting from rupture of a tuberculous false aneurysm of the aorta and to present a brief review of the literature.

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In 1933, Gellerstedt and Säfwenberg collected 20 cases of tuberculous aortic aneurysms and added 1 case of their own.* In these 21 cases, 12 instances of rupture were reported. In Table 1 these cases are listed, with a short description of the aneurysm

* The paper of Gellerstedt and Säfwenberg is used as source material covering the period prior to 1933.

TABLE 1.*—*Cases of Tuberculous Aortic Aneurysms up to 1933*

Case †	Author	Year	Age	Sex	Aneurysm	Associated Tuberculous Lesions
1 (2)	Kamen	1895	24	M	False aneurysm 4 cm. above aortic valve, produced by direct extension from a lymph node; rupture into pericardial cavity with tamponade	Chronic pulmonary & acute miliary tuberculosis
2 (6)	Vanzetti ‡	1908	--	--	False aneurysm of aortic arch produced by direct extension from paravertebral caseous mass; rupture occurred	No details
3 (8)	Zurnek	1914	27	F	False aneurysm in region of celiac artery, produced by direct extension from lymph node, rupture occurred	Chronic pulmonary; miliary tubercles in liver & spleen
4 (9)	Girardet	1914	30	M	False aneurysm 3 cm. above aortic valve, produced by direct extension from caseous node; rupture into trachea	Chronic apical pulmonary; miliary in lung, liver, kidney, testis, & pancreas
5 (10)	Edenhuizen	1915	34	M	False aneurysm of aorta near origin of subclavian artery, produced by direct extension from lymph node; rupture into esophagus	No details
6 (11)	Tozer ‡	1915	--	--	False aneurysm of abdominal aorta; rupture into duodenum	No details
7 (12)	Kornitzer	1920	17	M	False aneurysm 4 cm. above aortic valve; produced by direct extension from a lymph node; rupture occurred	Miliary in lung & kidney
8 (16)	von Zalka (Case 2)	1924	29	M	False aneurysm of sinus of Valsalva; produced by direct extension from lymph node; rupture into pericardium with tamponade	Early pulmonary
9 (17)	Dafoe (Case 1)	1925	34	M	True aneurysm in area of celiac artery, associated with false aneurysm; produced by direct extension from lymph node; rupture into peritoneal cavity	Tuberculosis infection of abdominal and thoracic organs and epididymis
10 (19)	Rosenthal	1930	82	F	False aneurysm of aortic arch; produced by direct extension from caseous node; rupture into trachea	Miliary in lungs
11 (20)	Clerici Bogazzi	1932	70	M	False aneurysm of abdominal aorta; produced by direct extension from lymph node; rupture into duodenum	Cavernous pulmonary tuberculous involvement of lumbar vertebrae
12 (21)	Gellerstedt & Säfwenberg	1933	16	M	False aneurysm 1 cm. proximal to left renal artery; produced by direct extension from lymph node; bleeding during surgery with resulting death	Miliary involvement of kidney and vertebrae L-4 and L-5 with a cold abscess

* From Gellerstedt and Säfwenberg.
† Number in parentheses is case number used by Gellerstedt.
‡ Taken by Gellerstedt from abstracts.

TABLE 2.—*Cases of Tuberculous Aortic Aneurysm Since 1933* *

Case	Author	Year	Age	Sex	Aneurysm	Associated Tuberculous Lesions
13	Owens & Bass	1944	72	F	False aneurysm cephalad to celiac axis; produced by a hematogenous infection via vasa vasorum; rupture occurred; acid-fast positive; atherosclerosis	Miliary tuberculosis
14	Scott et al.	1949	49	M	False aneurysm immediately cephalad to celiac axis; produced by direct extension from lymph node; rupture into stomach; acid-fast negative; no mention of atherosclerosis	Bilateral chronic apical pulmonary tuberculosis; miliary spread in liver & spleen; lymphadenitis
15	Brooks & Dawson	1949	45	M	False aneurysm of descending aorta; produced by hematogenous infection; rupture into esophagus; acid-fast positive; no mention of atherosclerosis	Bilateral apical pulmonary fibrocaseous tuberculosis
16	Bagnuolo & Bennett	1950	55	M	False aneurysm of thoracic aorta; produced by direct extension from lymph node; rupture into right lower lung & esophagus; no acid-fast report; no mention of atherosclerosis	Generalized miliary
17	Froehlich	1951	38	F	False aneurysm of thoracic aorta; produced by direct extension from lymph node; rupture into esophagus; no acid-fast report; atherosclerosis	Bilateral pulmonary tuberculosis with cavitation on left
18	Herndon et al.	1952	69	M	False aneurysm over aortic bifurcation; produced by direct extension from surrounding tissue; bleeding from aneurysm during operation; acid-fast positive; atherosclerosis	Miliary pulmonary tuberculosis; prostatic involvement
19	Sandrock et al.	1952	46	F	False aneurysm over bifurcation of aorta; produced by direct extension from lymph node; rupture into first part of jejunum; no acid-fast report; atherosclerosis	Advanced pulmonary tuberculosis with bilateral cavitation; peritonitis; tubercles in liver, spleen, & kidney
20	Malagoni	1953	48	F	False aneurysm at level of 1st intercostal artery; produced by direct extension from tuberculous pleuritis; rupture into left lung; no mention of atherosclerosis; no acid-fast report	Limited pulmonary miliary lesions; lymphadenitis of mesenteric & perisortic nodes
21	Simpson & Grobbelaar	1955	35	M	Rupture of thoracic aorta into paravertebral muscles with aneurysm formation; produced by direct extension from contiguous tissue; rupture into left pleural cavity; acid-fast positive; no mention of atherosclerosis	Miliary tuberculosis; involvement of vertebra T-12
22	Rothfeld et al.	1956	58	M	False aneurysm 3 cm. above bifurcation; produced by direct extension from contiguous tissue; rupture into duodenum; acid-fast positive; no mention of atherosclerosis	Tubercles in liver & spleen; left nephrectomy 3 yr. previously because of tuberculosis
23	German & Green	1956	36	M	False aneurysm 1 cm. below renal artery; produced by direct extension from lymph node; retroperineal rupture; acid-fast positive; no extensive atherosclerosis	Left apical pulmonary; tubercles in liver, spleen, left epididymis; lymphadenitis
24	Wettland & Scott	1956	51	M	False aneurysm of thoracic aorta; produced by extension from lymph node; rupture into left lower lobe of lung; cultures acid-fast positive; minimal atherosclerosis	Primary pulmonary tuberculous; paravertebral abscess at T-5
25	Foley et al.	1956	6½	M	False aneurysm of noncoronary aortic sinus; produced by direct extension from tuberculous pericarditis; cultures of pericardium positive; rupture during surgery	Pulmonary; scattered tubercles in liver; hematogenous infection of pericardium
26	Vahia	1956	26	F	False aneurysm of aorta at level of inferior mesenteric artery; produced by direct extension from lymph node; rupture occurred; acid-fast positive; atherosclerosis	Tuberculosis of left kidney; lymphadenitis
27	Stiefel	1957	44	F	False aneurysm 2 cm. proximal to aortic bifurcation; produced by direct extension from lymph node; rupture into peritoneal cavity; acid-fast positive; atherosclerosis	Primary complex; acute generalized miliary tuberculosis; bony involvement of L-4 & L-5; cold abscess of left psoas muscle

* Cases numbered in series with those of Table 1.

and the associated tuberculous lesions. In the remaining nine cases rupture was not reported; therefore, these cases are not included in Table 1.† Although no mention of rupture was made in the second case of Dafoe, the sudden death of the patient suggests that the aneurysm may have ruptured. The case is, however, not included.

Table 2 contains a list of 14 cases of ruptured tuberculous aortic aneurysms reported since 1933. The case to be presented here is included as the 15th one in Table 2. Included in Table 2 are the results of acid-fast stains for Mycobacterium tuberculosis, and mention is made of the presence of atherosclerosis.

It is necessary to point out why certain cases have not been included in this series, which is limited to tuberculous aneurysms with rupture. Aside from the 21 cases of aneurysm collected by Gellerstedt, 8 cases of tuberculous rupture of the aorta, without aneurysm formation, were reported in the same paper. These were cases of Barety,‡ Soloman, Doehle (1907), Haban (1932), Schnyder (1918), von Zalka (1924), and Schmorl (two cases, 1902). Four additional cases of tuberculous aortic rupture without aneurysm formation have been reported:

1. The case of Ferrabouc and Fauvert (1931)—an ulcerative tuberculous lesion of the esophagus eroded directly into the aorta without aneurysm formation.

2. The case of Frosch and Horowitz (1944)—a caseous lymph node eroded into the lower one-third of the duodenum and into the aorta, forming a duodenoaortic fistula.

3. The case of Zarowitz and Grayzel (1949)—a case of hematogenous tuberculous infection of the aorta with rupture into the third portion of the duodenum without aneurysm formation.

4. The case of Ghosh (1954)—a case of mesenteric adenitis in which a caseous lymph node eroded through the aortic wall and the duodenal wall.

The cases of Somerville and Wishart (1948) have not been included in this listing. Their first case was that of a 22-year-old woman who had Pott's disease involving the eighth thoracic vertebra. There was pathologic fracture of the eighth rib, with subsequent puncture of the aorta by the bone fragment. The second case was that of a 37-year-old man. An aneurysm which had ruptured into the left pleural cavity was found at autopsy. This case is not included because there is a definite question of a syphilitic etiology.

In the case of Rob and Eastcott (1955), a tentative diagnosis of lymphadenitis with aneurysm formation was made. At operation a tuberculous aneurysm of the aorta was found and replaced by an Orlon prosthesis. The case has, therefore, not been included, since the aneurysm was discovered and successfully repaired before rupture occurred. This is the only case in which the diagnosis was made prior to death and treatment was carried out.

Report of Case

A 44-year-old obese Negro woman was admitted to the John Gaston Hospital, the teaching hospital of the University of Tennessee, on April 6, 1957, in an irrational state. The patient's sister-in-law acted as informant.

The chief complaints were headache, back ache, stomach ache, irrationality, and severe lethargy.

The acute phase of her illness began nine days prior to admission, when she began to complain of anorexia and progressive lethargy. The family noticed that the patient was intermittently irrational, with increasingly shorter lucid intervals until the time of admission. About six days prior to admission the patient suffered from severe abdominal cramping pains.

The history of her illness dates back one year before her admission, at which time she quit her work as a cook because of pains in her right knee, right wrist, and right shoulder. These pains were described as feeling as if "pins were being driven in the bones and joints." There was no swelling, redness, or limitation of motion in the affected joints, although she complained of early-morning stiffness which decreased with motion. Other complaints reported include severe headache (for which she took one box of acetylsalicylic acid tablets per day), weight loss of 75 lb., progressive loss of visual acuity, paroxysmal nocturnal dyspnea, two-pillow orthopnea, and some exertional dyspnea. She also suffered from hypertension, for which

†Cases of Dittrich (1888), Hanau and Sigg (1896), Councilman and Mallory (1896), Liefmann (1904), Ribbert (1914), Benda (1900), Lenoble (1922), and Dafoe (Case 2, 1925).

‡Barety and Soloman quoted by Gellerstedt from Girardet (1914).

she was treated by a local physician, night sweats, intermittent fever of undetermined degree, nocturia (two times), occasionally dysuria, and low back pain. The patient's habits included use of tobacco and alcohol.

Upon admission physical examination revealed her pulse rate to be 140 per minute; blood pressure, 112/80 millimeters of mercury; respirations, 30 per minute, and temperature, 99 F. In general she was a well-developed obese Negro woman, disoriented but in no pain.

Examination of the optic fundi revealed a Grade III retinopathy (Keith-Wagner). The lips and oral mucosa were dry, and an area of leukoplakia was noted on the buccal mucosa opposite the right third molar. The neck was supple. Squeaking rales were heard throughout both lung fields. The apex beat was identified 2 cm. lateral to the midclavicular line in the fourth intercostal space.

The abdomen was markedly distended, and a questionable fluid splash was present. There was generalized tenderness to deep palpation, and slight muscle guarding was present.

The back was obese, straight, supple, and nontender.

Examination of the extremities revealed no evidence of inflammation, limitation of motion, or pain on passive motion. The deep tendon reflexes were hyperactive bilaterally, but no pathological reflexes were elicited.

Laboratory examination on admission revealed anemia, hematocrit of 27 vol.% and leukocytosis, with 10,500 white blood cells per cubic millimeter and a shift to the left (Schiller).

Urine examination revealed Gram-positive diplococci and rods in chains and Gram-negative rods. Further laboratory examination included a test with P. P. D. No. 1, which was negative; a histoplasma skin test, which was 1+, and several negative L. E.-cell preparations. Tests for urinary Bence Jones protein were negative, and agglutinations for typhoid, tularemia, Proteus X19, and undulant fever were negative.

Peritoneal aspiration was done, and a total of 7 cc. grossly bloody fluid was withdrawn, examination of which revealed no malignant cells or acid-fast bacilli. Repeated sputum examinations were negative for acid-fast bacilli. A posteroanterior film of the chest revealed a large calcified hilar lymph node and linear plate-like atelectasis, although no definite consolidation or infiltration were seen.

The VDRL test was negative.

Her treatment while in the hospital consisted of symptomatic and supportive measures. The patient died at 1:50 p. m. on April 12, 1957.

Autopsy Findings.—At autopsy the body was that of a well-developed well-nourished Negro woman.

When the peritoneal cavity was opened, approximately 1000 ml. of grossly bloody fluid escaped. An additional 500 ml. of grayish-red friable clot was found. All peritoneal surfaces were covered with a gray friable shaggy exudate, and there were numerous fibrous and fibrinous adhesions among the loops of bowel.

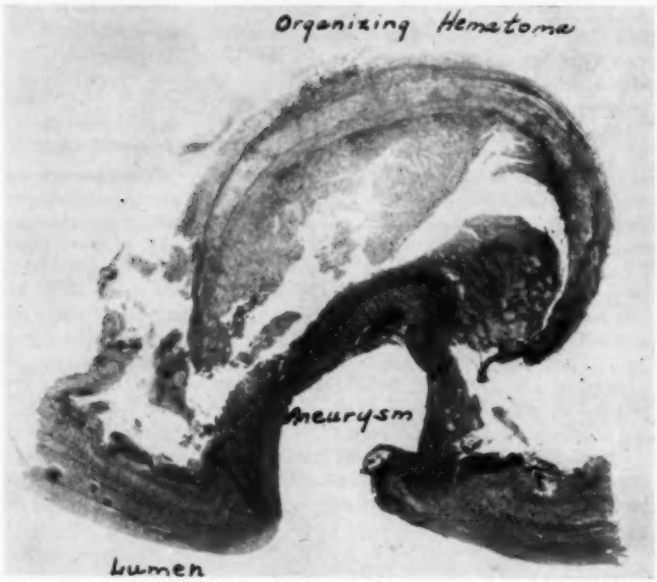
After removal of the intestines, a 5 cm. mass overlying the aortic bifurcation was noted. This was an intraperitoneal current-jelly clot associated with a retroperitoneal hematoma in the adjacent area. Removal of this clot revealed a 1.0 cm. false aneurysm of the aorta situated 2 cm. proximal to the aortic bifurcation. The aneurysmal wall was composed of grayish-white fibrous tissue and caseous necrotic material. Adjacent to the left common iliac artery a caseous lymph node, which had eroded through the wall and intima of the vessel, was noted. Although no discrete lymph node could be demonstrated about the aortic lesion, it is felt that the mycotic aneurysm was produced by destruction spreading from an adjacent lymph node, as was the case seen in the iliac artery.

The bodies of the fourth and fifth lumbar vertebrae were soft and necrotic. In the left psoas muscle a spindle-shaped cold abscess was noted. This abscess measured 10 cm. in greatest length and communicated with the vertebral lesion.

Other gross findings include evidence of a healed primary tuberculous complex in the lungs and very few firm encapsulated nodules in the spleen and liver.

On microscopic examination of the aorta, minimal atherosclerosis was noted. Sections taken from the aneurysm confirmed the presence of a false aneurysm composed of fibrous tissue and granulation tissue. Within and outside of the aneurysm sac were fragments of a laminated clot and masses of caseous material containing Langhans giant cells. Acid-fast bacilli were demonstrated with a Ziehl-Neelsen stain of the aortic aneurysm. No tissue characteristic of a lymph node was recognized. Sections of the left common iliac artery confirmed the presence of a tuberculous lymph node which had eroded through the entire wall and intima of the vessel.

Sections from the fourth and fifth lumbar vertebrae revealed a typical caseous osteolytic lesion. Examination of the psoas muscle abscess revealed a picture characteristic of tuberculosis, and acid-fast bacilli were demonstrated.



Photograph of aneurysm and the organizing clot about the defect; reduced about 45% from mag. × 10.

Examination of other organs revealed acute caseating miliary tubercles in the spleen, kidney, liver, peritoneum, brain, broad ligament, Fallopian tubes, myocardium, right adrenal, and lungs.

The pathogenesis of this case began when the patient contracted the typical primary tuberculous complex, consisting of subpleural tubercles and hilar lymphadenitis. From this lesion there was dissemination to the lower lumbar vertebrae, while the primary complex went on to healing and complete calcification. The disease process, however, continued in the vertebrae and gave rise to an abscess in the left psoas muscle and to periaortic lymphadenitis. The miliary spread of the disease is believed to have arisen from the erosion of a tuberculous node into the iliac artery, creating a macroscopic focus of discharge of large numbers of bacilli into the blood stream.[13] A second tuberculous lymph node eroded into the aortic wall, producing a mycotic aneurysm. The initial rupture is believed to have coincided with the woman's severe cramping abdominal pain six days prior to her hospital admission. She survived this episode, hence the antemortem clot found in and about the aneurysm. Survival for a number of days is not uncommon.[1,5,23] Finally, a second rupture, with ensuing fatal hemorrhage, occurred.

In 1924, von Zalka discussed three methods by which a tuberculous infection might invade the aorta: (1) hematogenous by way of the aortic blood stream, (2) hematogenous by way of the vasa vasorum, and (3) by direct extension from a neighboring focus of infection.

It is interesting to note that only three cases of hematogenous infection have been reported: those of Zarowitz and Grayzel (without aneurysm formation, 1949), Owens and Bass (1944), and Brooks and Dawson (1949). Table 3 shows that the commonest focus from which tuberculous infection has extended into the aorta is a lymph node.

Four cases of ruptured tuberculous aortic aneurysms have been associated with verte-

TABLE 3.—*Incidence of Infective Foci*

Infective Focus	Cases, No.
Lymph node	18
Unidentified tissues	5
Pleuritis	1
Pericarditis	1
Total	25

bral involvement. These are the cases of Clerici Bogazzi (1932), Gellerstedt and Säfwenberg (1933), Simpson and Grobbelaar (1955), and the present case. These aneurysms were produced by extension from contiguous tissues.

In three of the four cases the lumbar vertebrae were involved and associated with an abdominal aneurysm. In the fourth case the 12th thoracic vertebra was involved in association with a thoracic aneurysm. Other instances of Pott's disease in connection with rupture of the aorta without aneurysm formation have been reported. These include the first case of von Zalka (1924), the case of Schnyder (1918),§ and the first case of Somerville and Wishart (1948).‖

Summary

A review of the literature and a short survey of the 26 reported cases of ruptured tuberculous aortic aneurysms are presented.

A case of ruptured tuberculous aortic aneurysm is presented. This is the 27th case of this type and the 4th case of this type to be associated with Pott's disease of the spine.

Dr. T. P. Cruthirds assisted with this autopsy, Dr. Walter E. Stiefel, of the Department of Romance Languages, University of Tennessee, translated the paper by Malagoni, and Miss Jane Valerie King helped with the secretarial work.

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§ Cited by Gellerstedt.[7]

‖ The papers of Talantov and Vrcelj were not available at the time of this writing. They may or may not have some bearing on the subject discussed.

REFERENCES

1. Bagnuolo, W. G., and Bennett, H. D.: Nontraumatic Aortic Perforations into Gastrointestinal Tract: Review of the Literature and Report of an Unusual Case, Am. Heart J. 40:784-788 (Nov.) 1950.

2. Brooks, B., and Dawson, J. B., cited by Scott.[37]

3. Ferrabouc, L., and Fauvert, R.: Ulcération tuberculeuse primitive de l'œsophage: Perforation aortique, Ann. anat. path. 8:764-766 (July) 1931.

4. Foley, M. M.; Probert, W. R., and Seal, R. M. E.: False Aneurysm of Ascending Thoracic Aorta Complicating Tuberculous Pericarditis, Tubercle 37:783-786 (June) 1956.

5. Froehlich, W.: Anatomisch-pathologischer Beitrag der oralen Blutungen bei Lungenerkrankungen, Schweiz. Ztschr. Tuberk. 11:290-295, 1954.

6. Frosch, H. L., and Horowitz, W.: Rupture of Abdominal Aorta into Duodenum (Through a Sinus Tract Created by a Tuberculous Mesenteric Lymphadenitis), Ann. Int. Med. 21:481-485 (Sept.) 1944.

7. Gellerstedt, N., and Säfwenberg, O.: Zur Kenntnis der Aortatuberkulose und des tuberkulösen Arrosionsaneurysmas der Aorta, Upsala läkaref. förh. 38:165-181, 1933.

8. German, J. L., and Green, C. L.: Fatal Rupture of a Tuberculous Aortic Aneurysm: A Case Report, Ann. Int. Med. 45:698-702 (Oct.) 1956.

9. Ghosh, H.: Tuberculous Lymphadenitis: Report of a Case with Perforation of Aorta into Duodenum, Am. J. Clin. Path. 24:1044-1049 (Sept.) 1954.

10. Herndon, J. H.; Galt, J., and Austin, D. J.: Ruptured Tuberculous False Aneurysm of the Abdominal Aorta: Report of a Case with Resection of the Aneurysm and Survival for 6 Days, Texas J. Med. 48:336-338 (June) 1952.

11. Malagoni, C.: Su di un caso di rottura di un aneurisma tubercolare dell'aorta toracica, Riv. anat. pat. e onc. 6:725-732 (Feb.) 1953.

12. Owens, J. N., Jr., and Bass, A. D.: Tuberculous Aneurysm of the Abdominal Aorta: Report of a Case, Arch. Int. Med. 74:413-415 (Dec.) 1944.

13. Rich, A. R.: The Pathogenesis of Tuberculosis Ed. 2, Springfield, Ill., Charles C Thomas, Publisher, 1951.

14. Rob, C. G., and Eastcott, H. H. G.: Aortic Aneurysm Due to Tuberculous Lymphadenitis, Brit. M. J. 1:378-379 (Feb. 12) 1955.

15. Rothfield, B.; Garey, J. L., and Twining, R. H.: Tuberculous Abscess Invading Aorta and Duodenum with Fatal Gastrointestinal Hemorrhage, Gastroenterology 30:958-961 (June) 1956.

16. Sandrock, R. S.; Emerson, G. L., and Emerson, M. S.: A Rare Complication of Tuberculous Mesenteric Lymphadenitis: A Case of Fatal Hemorrhage from Abdominal Aorta into Jejunum Through a Tuberculous Lymph Node, Am. Rev. Tuberc. 65:210-214 (Feb.) 1952.

17. Scott, J. W.; Maxwell, E. S., and Grimes, A. E.: Tuberculous False Aneurysm of the Abdominal Aorta with Rupture into the Stomach: A Case Report with Review of the Literature, Am. Heart J. 37:820-827 (April) 1949.

18. Simpson, T. V., and Grobbelaar, B. G.: Rupture of the Aorta Complicating Tuberculosis of the Spine, J. Bone & Joint Surg. 37B:614-617 (Nov.) 1955.

19. Somerville, E. W., and Wishart, J.: Pott's Disease of the Spine with Rupture of the Aorta, J. Bone & Joint Surg. 30B:327-332 (May) 1948.

20. Talantov, V. A.: Cases of Aortic Perforation in Tuberculous Spondylitis, Ortop. travm. protez. 17:57-58 (July-Aug.) 1956.

21. de Vahia, J. L.: Anévrysme de l'aorte abdominale d'origine tuberculeuse, Maroc méd. 35: 50-51 (Jan.) 1956.

22. Vrcelj, S., and Janći ć., M.: Hemorrhagic Syndrome During Tuberculosis, Srpski arh. celok. lek. 84:762-770 (June) 1956.

23. Wettland, P., and Scott, D.: Tuberculous Aortic Perforations: Review of the Literature and Report of a Case of False Aneurysm with Rupture into a Bronchus, Tubercle 37:177-182 (June) 1956.

24. von Zalka, E.: Zwei Fälle von tuberkulöser Aortenperforation, Arch. path. Anat. 251:685-698 (Feb.) 1924.

25. Zarowitz, H., and Grayzel, D. M.: Tuberculous Arteritis of the Abdominal Aorta with Rupture into the Third Portion of the Duodenum: Report of a Case, Am. Rev. Tuberc. 60:801-807 (Dec.) 1949.

The Histochemical Demonstration of Hyaluronic Acid

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The ability to demonstrate hyaluronic acid in tissue sections is a matter concerning which there is a lack of agreement in the literature. Two well-known methods for the demonstration of mucopolysaccharides have been used for this purpose, namely, the metachromatic staining reaction and the periodic acid-Schiff (P. A. S.) reaction.

Meyer (1947) stated that hyaluronic acid in a concentration of 1% would show a metachromatic reaction with toluidine blue but considered that "the concentration present in tissues is probably never high enough to contribute to the metachromatic staining."

Wislocki, Bunting, and Dempsey (1947), Altshuler and Angevine (1949), Asboe-Hansen (1950), and Pearse (1953) have all stated that hyaluronic acid can be demonstrated in tissue sections by the metachromatic staining reaction. However, Sylvén and Malmgren (1952) concluded after an intensive study that hyaluronic acid constituted a borderline case between metachromatic and nonmetachromatic mucopolysaccharides. They therefore stressed the difficulties imposed by the largely unknown factors of linkages between hyaluronic acid and other tissue components, the degree of polymerization, and the presence of other metachromatic material when any attempt is made to assess the reactions of hyaluronic acid and hyaluronates in tissues. Davies (1943, 1952) stated categorically that hyaluronic acid does not stain metachromatically. Persson (1953) considered that approximately 10 times the concentration of material was necessary to give a metachromatic reaction in the case of hyaluronic acid than was necessary with a comparable sulfated mucopolysaccharide and that the degree of polymerization of the acidic mucopolysaccharides (as expressed by their relative viscosities) did not justify any prediction as to their metachromatic potency.

There are similar differences of opinion as to whether hyaluronic acid can be demonstrated by the P. A. S. reaction or not. Hotchkiss (1948) stated that the reaction was very strong with hyaluronic acid, but Davies (1952) and Glegg, Clermont, and Leblond (1952) reported that sulfur-free preparations of hyaluronic acid gave no reaction. Pearse (1953) includes hyaluronic acid in his list of P. A. S.-positive materials in tissue sections.

In 1946, Hale described a method for hyaluronic acid and other acidic mucopolysaccharides in which colloidal ferric hydroxide is linked to the acidic groups of the mucopolysaccharide; the absorbed iron is demonstrated as Prussian blue by treatment with hydrochloric acid and potassium ferrocyanide. According to Hale (1946), the iron does not combine with proteins or neutral polysaccharides, but Lillie and Mowry (1949) and Pearse (1951) have shown that the binding of iron is not limited to acidic mucopolysaccharides, and Davies (1952), Gomori (1952), Persson (1953) and Braden (1955) consider that the method is not specific.

It seemed that hyaluronic acid might be demonstrable by the sulfation-metachromasia reaction (Kramer and Windrum, 1953, 1954). To test this, some studies have been carried out on the staining of vitreous humor as a source of native hyaluronic acid. The eye is a convenient organ in which to study the histochemical reactions of carbohydrate-containing complexes. In addi-

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tion to the vitreous humor, which contains no demonstrable ester sulfate (Meyer, 1950), there are complexes of acidic mucopolysaccharides in the cornea (Woodin, 1952), conjunctival mucous cells, mast cells, and ground substance about the vessels and the optic nerve. The lens capsule contains at least 10% carbohydrate (Pirie, 1951), and the vitreous residual protein contains appreciable amounts of carbohydrate (Pirie, Schmidt, and Waters, 1948; Young and Williams, 1954).

Material and Methods

Fresh eyes removed from rabbits of various ages which had been killed by pentobarbital (Nembutal) anesthesia were used.

Smears of fresh vitreous humor from 6-week-old rabbits were made on clean glass slides and dried in an incubator at 37 C for two hours. The dried smears were fixed in absolute alcohol, Carnoy's, formol-saline, Zenker-acetic, or Bouin's fixatives for five hours. They were briefly rinsed in absolute alcohol and redried in the incubator at 37 C prior to staining by the normal metachromatic reaction (Kramer and Windrum, 1955), P. A. S. test (McManus, 1948, first reference) and sulfation-metachromasia reaction (Method 2 of Kramer and Windrum, 1954).

A 6-week-old rabbit eye was fixed in Carnoy's fluid, and another, in formol-saline for 12 hours; paraffin sections were prepared and stained similarly to the vitreous smears. Some lenses from rabbits were similarly treated, as were a number of whole eyes from rabbits of various ages, including fetal eyes.

Some deparaffinized sections were incubated in testicular hyaluronidase (Rondase, Evans), 100 mg. in borate buffer pH 6.8 for 12 hours, prior to staining. Control sections were incubated in the buffer alone and in a heated solution of the enzyme preparation.

Results

Staining of the fixed smears by the normal metachromatic method showed some areas of alcohol-fast metachromasia where the smear was thick but only faint orthochromatic staining where the smears were thin. The P. A. S. reaction gave a faint pink coloration without any areas showing a strongly positive reaction. The sulfation-metachromasia reaction gave a pronounced red coloration of the whole smear.

With the sections from the fixed eyes, the normal metachromatic staining reaction showed only a faint orthochromatic staining of the vitreous, while there was metachromatic staining of the corneal ground substance, mast cells in the iris, mucous cells of the conjunctiva, and some material in the walls of blood vessels and the sheath of the optic nerve. The P. A. S. reaction showed a faint pink color in the vitreous, a moderate reaction in the corneal ground substance, and a bright red reaction of the lens capsule, ciliary fibers, and conjunctival mucous cells.

The sulfation-metachromasia reaction showed an obvious metachromasia in the vitreous and in the stroma of the iris. The lens capsule and ciliary fibers were bright red. The normal metachromasia of the cornea, mast-cell granules, and blood vessel walls was not significantly altered; that of the conjunctival mucous cells was increased somewhat. There did not appear to be any significant difference between the eyes fixed in Carnoy and those fixed in formol-saline, though Hale (1946) has said that aqueous fixatives should not be used if it is wished to preserve hyaluronic acid. Pearse (1953) considers formalin an adequate fixative for mucopolysaccharides. No special precautions were taken to preserve the material during the preparation of these sections.

The Table shows the reactions of the principle carbohydrate components in the sections.

In the eye sections incubated in testicular hyaluronidase prior to staining, the normal metachromasia remained in the corneal ground substance, mast-cell granules, and conjunctival mucous cells, but the normal metachromasia previously seen in the walls

Reactions of Principal Carbohydrate Compounds in the Sections

Section	P. A. S.	Normal Metachromasia	Sulfation-Metachromasia
Corneal ground substance	Moderate positive	Metachromatic	Metachromatic
Lens capsule	Strong positive	Orthochromatic	Metachromatic
Ciliary fibers	Strong positive	Orthochromatic	Metachromatic
Iris stroma	Negative	Orthochromatic	Metachromatic
Vitreous	Negative	Orthochromatic	Metachromatic

of blood vessels and the optic nerve sheath was lost. Woodin (1952) has isolated a number of sulfated mucopolysaccharides from cornea which are not hydrolyzed by hyaluronidases. Wislocki, Bunting, and Dempsey (1947) have recorded that the metachromasia of cornea is not removed by digestion with hyaluronidase. With the sulfation-metachromasia reaction, the corneal ground substance, mast-cell granules, conjunctival mucous cells, lens capsule, and ciliary fibers retained their positive reaction in spite of the hyaluronidase digestion, but the reaction of the stroma of the iris was reduced considerably, as was that of the vitreous. The control sections treated with heated enzyme solution and buffer prior to staining also showed a slight loss of reactive material in these two sites but not nearly as great a reduction as in the enzyme-digested sections. Examination of the enzyme-digested slides showed that the remaining sulfation-metachromasia-positive material in the vitreous was present in fibrillar form. This suggested that this may be due to a reaction of the residual protein. Examination of the eyes of rabbits less than 10 days old and fetal eyes showed that the vitreous gave a positive reaction to the sulfation-metachromasia reaction, although Bembridge and Pirie (1951) have shown that hyaluronic acid does not appear in rabbit eyes until 10 days after birth. These results suggested that the residual protein contained carbohydrate which could not be removed by hyaluronidase but which was capable of giving a positive sulfation-metachromasia reaction. Pirie, Schmidt, and Waters (1948) found that the residual material after removal of all hyaluronic acid from ox vitreous contained 3% to 7% carbohydrate, and Young and Williams (1954) reported that the residual protein after incubation of ox vitreous in hyaluronidase contained 4.3% carbohydrate, though no hexosamine was detected. This would indicate that the carbohydrate value was not due to a hyaluronic acid-protein complex but to a different type of glycoprotein complex. The fact that a positive sulfation-metachromasia reaction can be obtained in vitreous sections from eyes at an age before that at which hyaluronic acid can be detected biochemically also suggests that a glycoprotein complex is present even at this early stage in development. The protein component of this complex would appear to be of a collagen type, since Pirie, Schmidt, and Waters (1948) have shown that it is dissolved by a specific collagenase.

No attempt was made to determine quantitatively the amount of hyaluronic acid present in the vitreous smears, but it seems that one may deduce that hyaluronic acid from this source is only faintly P. A. S.-reactive, is very reactive to the sulfation-metachromasia reaction, and may show some normal metachromasia if the local concentration be high enough.

The study of the eye sections showed that though hyaluronic acid may not be present in sufficient concentration to be normally metachromatic or P. A. S.-positive it may still be present in sufficient quantity to be demonstrated by the sulfation-metachromasia reaction. The best demonstration was given by the chlorosulfonic acid-pyridine method (Method 2 of Kramer and Windrum, 1954).

Comment

The normal metachromatic staining reaction has been the subject of much discussion (Kramer and Windrum, 1955; Schubert and Hamerman, 1956). With the standardization of the technique to be used and use of adequate dyes, the problem of the reactivity of various substances depends upon knowledge of their concentration in tissue sections and their state of binding or association with other tissue elements.

Detailed investigation has been made of the P. A. S. reaction. The chemical basis of this (Malaprade, 1934) is that periodic acid will produce an oxidative cleavage of 1:2 glycol groups to produce two aldehyde groups. The aldehyde groups can then be demonstrated by their ability to produce a color with Schiff's reagent. Nicolet and

Shinn (1939) demonstrated that aldehyde can be produced by periodic acid from 1:2 glycols even if one hydroxyl group be substituted by an amino group, as in the amino acids serine, threonine, and hydroxyproline. However, since the necessary linkages are not free in the combined amino acids of tissue proteins and as the free amino acids are water-soluble, positive reactions are not likely to be produced in histological preparations by these substances (McManus, 1948, first reference).

The reaction was developed as a histochemical test by McManus (1946, 1948), Lillie (1947), and Hotchkiss (1948). According to Hotchkiss (1948), the following requirements must be satisfied if a positive reaction is to be obtained:

(*a*) Preservable material with the proper linkages must be present, i. e., 1:2 glycol groups or the equivalent amino or alkylamino derivative.

(*b*) The R.CHO groups formed must not be soluble, i. e., the aldehyde groups must be attached to an insoluble substance.

(*c*) The concentration of the final aldehyde-fuchsin compound must reach a certain level in the section to be visible.

Pearse (1953) lists hyaluronic acid as one of the positive materials in histological work, but many other workers, e.g., Davies (1952) and Glegg, Clermont, and Leblond (1952), do not agree with this. The negative reaction of purified hyaluronic acid samples reported by these authors is in agreement with the findings of Meyer and Fellig (1950), Jeanloz (1950), and Blix (1951) that hyaluronic acid consumes only small amounts of periodic acid. Jeanloz (1950) suggested that the glucosidic linkages of hyaluronic acid are of 1:3 type, and thus reactive glycol groups for periodic acid oxidation would not be available. According to Meyer and Fellig (1950), periodic acid attacks only the end-groups of the long-chain structure of hyaluronic acid, but Blix (1951) considered the periodic acid consumption to be too large to be explained on this basis and suggested that hyaluronic acid may possess alternating 1:3 and 1:4 linkages or an irregular structure varying with hyaluronic acids of different origins. Persson (1953), in reviewing the histochemical implications of these workers and investigating the reaction of various samples of hyaluronic acid, concluded that highly polymerized hyaluronic acids are hardly demonstrable by the P. A. S. reaction but that depolymerization, by increasing accessible 1:2 glycol groups, might increase this reactivity somewhat.

The results of the present investigation into the hyaluronic acid of rabbit vitreous suggest that it does not give a significant reaction with the P. A. S. test and that it is not normally metachromatic in the concentrations encountered in tissue sections. It would appear that the nature of the glycol linkages may not be suitable for periodic oxidation to aldehyde, or the reaction may be too slow, or the final aldehydes may be in insufficient concentration to give a color with the Schiff's reagent. Similarly the relative concentration of free carboxylic groups may not be high enough to induce normal metachromasia. However, Walton (1954) has shown that the metachromatic activity of acidic polysaccharides is determined by the presence and nature of their acidic groups and not by their degree of polymerization. Hence, if carbohydrate groups are available that can be sulfated (and this is probably largely independent of the configuration of the polysaccharide), then these groups may be demonstrable by the sulfation-metachromasia reaction. Some slight depolymerization may be caused by the acidic nature of the reagents used, but this would be of relatively minor importance (Persson, 1953; Walton, 1954). It would seem that sulfation may therefore demonstrate groups of mucopolysaccharides and mucoproteins that are not adequately shown by either the normal metachromasia or P. A. S. tests, and hyaluronic acid would appear to be one of these.

Summary

Some carbohydrate-containing complexes, such as lens capsule, may be demonstrated by both the periodic acid-Schiff (P. A. S.)

reaction and the sulfation-metachromasia reaction.

Other carbohydrate complexes, e.g., hyaluronic acid, may be negative to the P. A. S. reaction but positive to the sulfation-metachromasia reaction.

The low chromotrope potency of hyaluronic acid in tissue sections can be greatly increased by sulfation prior to staining with a metachromatic dye.

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BIBLIOGRAPHY

Altshuler, C. H., and Angevine, D. M.: Histochemical Studies on the Pathogenesis of Fibrinoid, Am. J. Path. 25:1061-1077, 1949.

Asboe-Hansen, G.: Survey of Normal and Pathological Occurrence of Mucinous Substances and Mast Cells in Dermal Connective Tissue in Man, Acta dermat.-venereol. 30:338-347, 1950.

Bembridge, B. A., and Pirie, A.: Biochemical and Histological Changes in Developing Rabbit Eyes, Brit. J. Ophth. 35:784-789, 1951.

Blix, G.: Structure of Hyaluronic Acid, Acta chem. scandinav. 5:981-989, 1951.

Braden, A. W.: The Reaction of Isolated Mucopolysaccharides to Several Histochemical Tests, Stain Technol. 30:19-26, 1955.

Davies, D. V.: Staining Reactions of Normal Synovial Membrane with Special Reference to Origin of Synovial Mucin, J. Anat. 77:160-169, 1943.

Specificity of Staining Methods for Mucopolysaccharides of the Hyaluronic Acid Type, Stain Technol. 27:65-70, 1952.

Glegg, R. E.; Clermont, Y., and Leblond, C. P.: The Use of Lead Tetraacetate, Benzidine, O-Dianisidine and a "Film Test" in Investigating the Periodic-Acid-Schiff Technic, Stain Technol. 27:277-305, 1952.

Gomori, G.: Microscopic Histochemistry: Principles and Practice, Chicago, The University of Chicago Press, 1952.

Hale, C. W.: Histochemical Demonstration of Acid Polysaccharides in Animal Tissue, Nature, London 157:802, 1946.

Hotchkiss, R. D.: Microchemical Reaction Resulting in the Staining of Polysaccharide Structures in Fixed Tissue Preparations, Arch. Biochem. 16:131-141, 1948.

Jeanloz, R.: Hotchkiss Reaction and Structure of Polysaccharides, Science 111:289, 1950.

Kramer, H., and Windrum, G. M.: Metachromasia After Treating Tissue Sections with Sulphuric Acid, J. Clin. Path. 6:239-240, 1953.

Sulphation Techniques in Histochemistry with Special Reference to Metachromasia, J. Histochem. 2:196-208, 1954.

The Metachromatic Staining Reaction, J. Histochem. 3:227-237, 1955.

Lillie, R. D.: Reticulum Staining with Schiff Reagent After Oxidation by Acidified Sodium Periodate, J. Lab. & Clin. Med. 32:910-912, 1947.

———and Mowry, R. W.: Biochemical Studies on Absorption of Iron by Tissue Sections, Bull. Internat. A. M. Museums 30:91-94, 1949.

McManus, J. F. A.: Histological Demonstration of Mucin After Periodic Acid, Nature, London 158:202, 1946.

Histological and Histochemical Uses of Periodic Acid, Stain Technol. 23:99-108, 1948.

The Periodic Acid Routine Applied to the Kidney, Am. J. Path. 24:643-653, 1948.

Malaprade, L.: Etude de l'action des polyalcools sur l'acide periodique et les periodates alcalins, Bull. Soc. chim. france (s. 5) 1:833-852, 1934.

Meyer, K.: The Biological Significance of Hyaluronic Acid and Hyaluronidase, Physiol. Rev. 27:335-359, 1947.

Chemistry of Connective Tissue: Polysaccharides, in Connective Tissues: Transactions of the First Conference, April 24-25, 1950, New York, edited by C. Ragan, New York, Josiah Macy, Jr. Foundation, 1950, p. 93.

———and Fellig, J.: La Constitution de l'acide hyaluronique, Experientia 6:186, 1950.

Nicolet, G. H., and Shinn, L. A.: The Action of Periodic Acid on β-Amino Alcohol, J. Am. Chem. Soc. 61:1615, 1939.

Pearse, A. G. E.: A Review of Modern Methods in Histochemistry, J. Clin. Path. 4:1-36, 1951.

Histochemistry: Theoretical and Applied, London, J. & A. Churchill, Ltd., 1953.

Persson, B. H.: Studies on Connective Tissue Ground Substance: Histochemical Features of Ground Substance Mucopolysaccharides; Organization of Ground Substance in Ascorbic Acid Deficiency and Its Modification by Action of Cortisone, Acta Soc. med. upsal. (Supp. 2) 58:3-104, 1953.

Pirie, A.: Composition of Ox Lens Capsule, Biochem. J. 48:368-371, 1951.

———Schmidt, G., and Walters, J. W.: Ox Vitreous Humour: I. The Residual Protein, Brit. J. Ophth. 32:321-339, 1948.

Schubert, M., and Hamerman, D.: Metachromasia: Chemical Theory and Histochemical Use, J. Histochem. 4:159-189, 1956.

Sylvén, B., and Malmgren, H.: On the Alleged Metachromasia of Hyaluronic Acid, Lab. Invest. 1:413-431, 1952.

Walton, K. W. W. H.: Histochemical Investigation of the Polymerization of Acidic and Neutral Polysaccharides, Proc. Roy. Soc. Med. 47:88-89, 1954.

Wislocki, G. B.; Bunting, H., and Dempsey, E. W.: Metachromasia in Mammalian Tissues and Its Relationship to Mucopolysaccharides, Am. J. Anat. 81:1-37, 1947.

Woodin, A. M.: The Corneal Mucopolysaccharide, Biochem. J. 51:319-330, 1952.

Young, R. G., and Williams, H. H.: Biochemistry of Eye: Gelatinous Protein of Vitreous Body, A. M. A. Arch. Ophth. 51:593-595, 1954.

Aortic Elastic Tissue

Isolation with Use of Formic Acid and Discussion of Some of Its Properties

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Various techniques for the isolation and characterization of elastic tissue have been proposed since Eulenberg, as quoted by Schultze,[19] first discovered in 1836 that gelatin could be obtained from the media of arteries by treatment with boiling water. The basic principle in all was to dissolve elements of connective tissue, with the exception of the more inert elastica. Collagen, the most resistant element, was removed by being converted to water-soluble gelatin with heat. Hot water alone was advocated by Vandegrift and Gies,[72] Stein and Miller (first method),[71] Bowes and Kenten,[13] Neuman and Logan,[59] Neuman (first method),[58] and Partridge et al.[1,61] Hot dilute acetic acid was first suggested by Tilanus, in 1844, and has since been used by Müller, Horbaczewski (Tilanus, Müller, and Horbacewski cited by Richards[63]), Chittenden and Hart,[15] Richards and Gies,[63] Gross,[23] Hall et al.,[26-31] Banga et al.,[2-8] and Bowen.[12] Hot dilute alkali, suggested by Müller, in 1861, and discarded by Chittenden and Hart, in 1887, as being destructive of the elastic tissue, was readvocated by Schwarz,[67] Schepilewsky,[66] Lowry et al.,[50] Lansing et al.,[18,42-46] and Banga et al.[2-8]

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In 1942, Hass proposed the use of concentrated formic acid for the isolation of aortic elastic tissue.[34] This followed the studies by Loiseleur, who found that anhydrous formic acid had a selective solvating action upon various proteins.[21,51] It differs thus from other methods by being effective without the presence of water. Many morphological, physiological, and chemical properties of elastic tissue have resulted from studies of whole aorta and ligamentum nuchae and purified elastica isolated from them by the various methods.[10,11,14,17,18,20,33,73]

In the present study it is intended to proceed with the critical evaluation, instigated by Hass, of the effect of concentrated warm formic acid upon whole aorta, expanding upon and clarifying any distinctive properties of elastic tissue revealed by the procedure. The plan is subdivided into two main parts: the attempt to prove (1) that elements other than elastic tissue are removed by the use of the acid and (2) that the purified elastin thus obtained is not significantly altered from the original elastic tissue. The first part only is presented in this study.

Isolation of Elastic Tissue from Whole Aorta

General Methods

The aortae of dogs are chosen for study because of their convenient size and accessibility and because the canine vascular system has been well investigated by surgical research.

Five tests to demonstrate the removal of elements other than elastic tissue from the aortic wall during exposure to warm concentrated formic acid are utilized:

1. Morphological examination, including electron microscopy.

2. Determination of the rate of loss of weight.
3. Determination of changes in mechanical elasticity (reversible extensibility).
4. Determination of changes in chemical elasticity (reversible dimensional changes caused by formic acid).
5. Determination of changes in thermal elasticity (reversible dimensional changes caused by changes in temperature).

General Procedures

Two hundred one similar rings are excised from the thoracic aortae, between the subclavian and the eighth intercostal arteries, of 18 qualitatively young healthy dogs. After removal of adventitial tissue by sharp dissection the rings have an average size of 0.7 cm. in diameter, 0.35 cm. in width, and 0.1 cm. in thickness. They are divided into five groups for the serial application of the tests at regular intervals of time during their prolonged exposure to warm concentrated formic acid. The exposure is accomplished in the following manner. After being rinsed in distilled water the rings are blotted and placed individually in sealed glass vessels containing at least 1 ml. of 88% formic acid (Merck) for each 5.0 mg. of dry tissue. The vessels are maintained at a temperature of 45±0.5 C in a constant temperature bath for regular periods of time. Whenever possible the same ring is used for multiple serial tests. Owing to irreversible alteration of the collagen and muscle caused by the tests this cannot be done in the early, or extractive phase, of exposure of the tissue to formic acid, adjacent rings being used instead. However, after the extractive phase of exposure to the acid no alteration of the tissue is found to be caused by multiple tests, and thus the same ring can be repeatedly treated and tested in a serial manner.

In order to compare and contrast the effect of formic acid upon collagenous tissue, strips of Achilles tendon from the same dogs are tested in a similar way. These are obtained by separation of the tendon into its longitudinal components by blunt dissection along natural lines of cleavage. They have an average length of 2.5 cm. and diameter of 0.3 cm.

Smooth muscle from the uterus, striated muscle from the hamstring muscles, and fat from subcutaneous depots are used to test qualitatively the effect of formic acid on muscular and fatty tissues.

1. Morphological Examination, Including Electron Microscopy

Methods

Fifty-six rings of aorta are used for the morphological evaluation of the extractive effect of concentrated formic acid upon aortic tissue. Seven rings are used for control study; the remainder are treated with 88% formic acid at 45 C for periods of time of 3, 6, 12, 24, 36, 72, and 216 hours. At the end of each period a group of seven rings is removed from the acid and washed to neutrality with running tap water. In each group four rings are left unfixed. One is saved for electron microscopy; one is used for cutting cross sections; one is opened for cutting sagittal sections; the last is opened and pinned flat on a cork board in a state of 30% extension for cutting tangential sections. The remaining three rings in each group are fixed with 10% neutral formalin before cutting cross, tangential, and sagittal sections in the same manner used for the unfixed material. With the exception of the ring to be used for electron microscopy all specimens are rapidly dehydrated with ethyl alcohol, cleared, and embedded with paraffin. Sections are cut with thicknesses between 1μ and 6μ. They are stained with the following stains: hematoxylin and eosin, Verhoeff's elastic-tissue stain with a Van Gieson counterstain, Mallory's aniline blue-orange G connective-tissue stain, Kornhauser's quad stain,[59,60] Gomori's aldehyde-fuchsin stain[61] and the Schiff stain with and without prior oxidation with periodic acid as described by McManus.[64]

Cross and longitudinal sections of Achilles tendon are handled in a manner identical to that used for aortic tissue.

For purposes of electron microscopy the control ring and the unfixed rings exposed to formic acid for the varying periods of time are cut into two parts for fragmentation and dispersion with a microblender. One part is suspended in 3 ml. of McIlvaine's buffer (pH 5.6; μ 0.15); the other in 3 ml. of 88% formic acid at 25 C. It is found that it is impossible to disperse aorta in a manner suitable for electron microscopy, regardless of the suspending medium, until the time of its exposure to concentrated formic acid at 45 C amounts to 36 hours or more. After an exposure of 36 hours, tissue suspended in buffer, when blended, breaks down into amorphous particles and debris too dense for electron microscopy, whereas tissue suspended in the formic acid results in excellent dispersion and separation of the fibrillar networks composing it. One drop of the latter suspension is applied to each of two glass slides. One of these is blotted and quickly air-dried; the other is neutralized by repeatedly adding and blotting away buffer followed by rinsing in water and drying in air. The remainder of the suspension in formic acid is centrifuged at 1000 rpm for five minutes to remove larger fragments. One drop of the supernate is applied to each of two glass slides and each of two nickel-plated collodion-coated electron microscopic screens. The drops on the glass slides are treated as before, one being dried

Fig. 1.—Tangential section of whole control canine aorta. Ribbons of elastic tissue caused by the plane of section passing through the undulating fenestrated lamellae contrast with collagen and smooth muscle. Verhoeff-Van Gieson stain; reduced 15% from mag. × 250.

of formic acid and the other being neutralized by buffer before drying. The drops on the two electron microscopic screens are treated in identically the same manner except that 1% phosphotungstic acid is used to flood the films for one minute immediately before rinsing and drying. The screens are then shadowed with chromium at an angle of 14 degrees and a depth of 10 A. and visualized with an RCA EMU-2 Electron Microscope.

Results

In tangential sections of whole untreated aorta the elastic tissue appears as broad, dark, fenestrated ribbons (Fig. 1). These are roughly parallel and have wavy distinct margins at right angles to the fiber axis. They occur on a separate plane from the cells and stroma of the collagenous and muscular tissues, with only extremely fine fibrillar attachments to them. The ribbon-formation, caused by the plane of section passing through incompletely flattened undulations of lamellae, is found to be advantageous because it allows visualization of the latter at a variable thickness and an evaluation of their relationship to other elements in the aortic wall. In areas representing increased or full thickness of the lamellae the ribbons are composed of a refractile, heavily stained, nearly homogeneous, membranous matrix in which occur round to oval fenestrations varying between 8μ and 26μ

Fig. 2.—Cross section of whole control canine aorta. The familiar undulating pattern of elastic tissue represents the cut edges of the lamellae seen in Figure 1. Verhoeff-Van Gieson stain; reduced 40% from mag. × 125.

in diameter. Branching interconnected bands and fibers of elastic tissue bridge the gap between ribbons in several places, indicating their true continuity with each other as a single broad sheet. In areas representing decreased or partial thickness of lamellae the fenestrations are of larger size and more oval, with long axes running predominantly in a circumferential direction. Between and outlining them occur bands of elastic tissue of varying width. These split about the fenestrations only to fuse again and join with neighboring bands to form even broader bands. In areas representing the thinnest material a longitudinal striation and even distinct formation of fibers surrounding smaller fenestrations is seen to occur within the bands. In the same areas many of the larger fenestrations are crossed irregularly by fine branching anastomotic fibers resulting in subloculation.

The relationship of collagen and muscle to the fenestrations is not clear. During staining of unfixed material under direct observation shrinkage of the broad lacework of the elastic lamellae occurs. The finely frayed margins of the ribbons are seen to lift from attachments with the underlying stroma, the ribbons themselves contracting to denser, narrower, and more evenly contoured structures in which the finer inner meshworks of bands and fibers become fused and obscured. The larger fenestrations diminish in size and assume rounder more clearly defined margins as the surrounding matrix becomes denser and more homogeneous. In some sections closely applied fine lamellae with long axes of fenestrations running obliquely or at right angles to each other are seen.

Cross sections of aorta show the familiar concentric undulating lines of elastic tissue representing the cut edges of the lamellae (Fig. 2). These are separated by the cells and stroma of collagen and smooth muscle and are interconnected by oblique radial fibers. Careful examination of unfixed, weakly stained, extremely thin sections (less than 2μ) reveals defects and irregularities in the lamellar edges suggesting the points of separation and contact of the branching bands and fibers about the fenestrations seen in tangential sections. In addition it appears that each lamella is composed of multiple, closely applied, thinner sheets. The outermost and innermost of these split away at irregular intervals around the circumference to fuse with contiguous lamellae, thus forming a lamellar syncytium. Sagittal sections of aorta show less undulation of the edges of elastic lamellae. These, in weakly stained thin sections of unfixed material, have an ill-defined beaded appearance resulting from visualization of the cut ends of bands and fibers of elastic tissue.

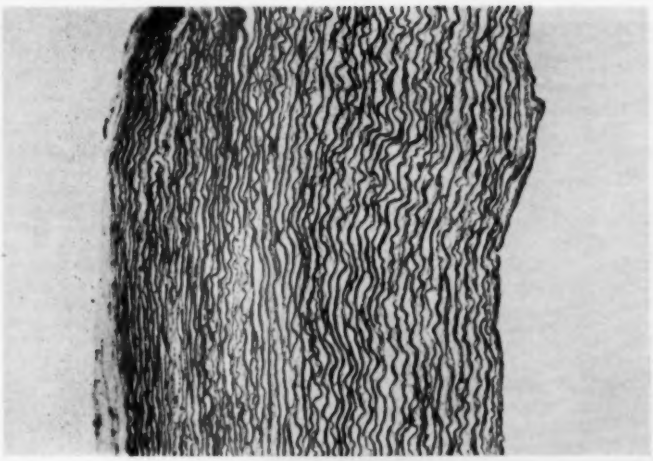

Fig. 3.—Cross section of purified elastica isolated with formic acid. Collagen, muscle, and cells have dissolved, leaving only the purple-stained elastic lamellae. These are slightly widened, revealing the suggestion of an inner laminar structure. Anastomotic connections between lamellae are visible. The total thickness of the wall is reduced to about one-half that of whole aorta. Verhoeff-Van Gieson stain; reduced 40% from mag. × 125.

Fig. 4.—Tangential section of purified elastica isolated with formic acid. All elements are stained purple without evidence of collagen, muscle, or cells. The broad lamellae are composed of band-like fibrillar aggregates of varying size, anastomosing about fenestrations of varying size. Verhoeff-Van Gieson stain; reduced 15% from mag. × 250.

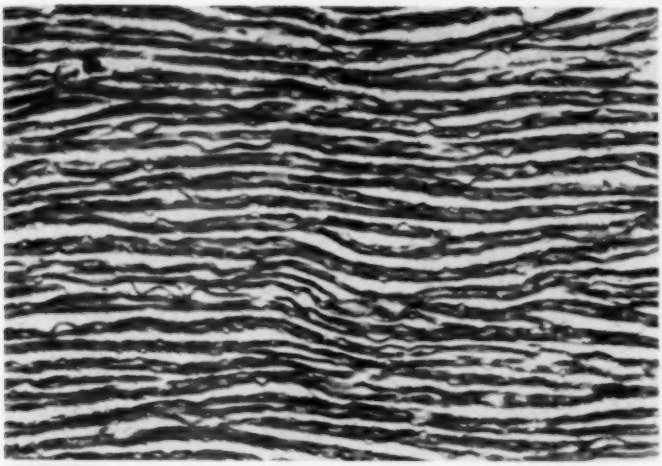

Fig. 5.—Extremely thin lightly stained cross section of unfixed purified elastica. The inner laminated structure within the lamellae and their anastomotic interconnections is demonstrated. Gomori's aldehyde-fuchsin; reduced 40% from mag. × 500.

Sections of whole aorta stained by the Method of McManus confirm the findings that elastic lamellae and fibers are sheathed by a thin film of reducing substance released only by previous oxidation with periodic acid.[16,70]

Exposure of the aortic tissue to 88% formic acid at 45 C results in marked swelling, hyalinization, and loss of special staining characteristics of the muscle and collagen within the initial period of three hours. The sheathing of elastic elements by reducing substance disappears between 6 and 12 hours. All evidence of collagen, muscle, and cells vanishes between 18 and 24 hours, unveiling with greater clarity the structure of elastic tissue, which retains unaltered its special staining characteristics (Fig. 3). In tangential sections the ribbons of the purified elastic tissue are broader and in extensive areas are continuous, forming infinitely broad sheets (Fig. 4). The bands, fibers, and fenestrations remain fundamentally the same as before exposure to formic acid, but finer subdivisions of a similar arrangement are now distinctly seen within them. In many areas extremely thin lamellae, with long axes of fenestrations running in different directions, appear to be closely applied to each other. In thin and

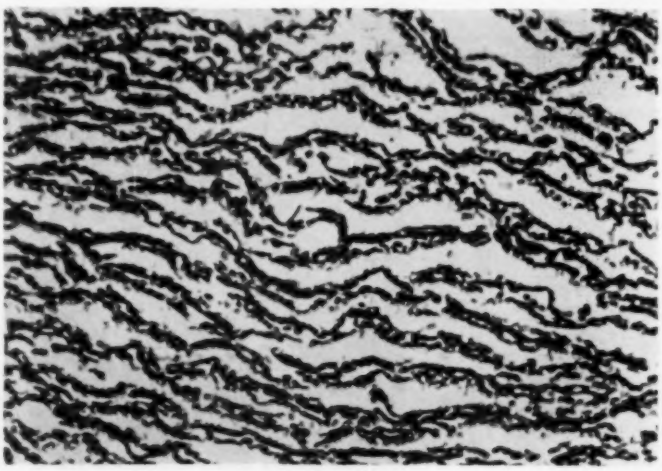

Fig. 6.—Extremely thin lightly stained sagittal section of unfixed purified elastica. That the fibrillar aggregates run predominantly in a circumferential direction is determined by the beaded appearance of their cut ends in this plane of section. Gomori's aldehyde-fuchsin; reduced 40% from mag. × 500.

Fig. 7.—Blended suspension of purified elastica, fully extended by concentrated formic acid. The fenestrated lamellae have fractured to form plates. These are composed of band-like fibrillar aggregates of varying size, anastomosing about fenestrations of varying size. Thicker plates are more homogeneous. Gomori's aldehyde-fuchsin; reduced 40% from mag. × 125.

lightly stained cross sections of unfixed extracted aorta the defects within the edges of lamellae caused by fenestrations and the occurrence of an inner laminated structure are now clearly seen (Fig. 5). In thin and lightly stained sagittal sections of unfixed extracted aorta the lamellae appear predominantly as rows of oval or round dots, representing the cross sections of bands and fibers (Fig. 6).

Corresponding to the loss of collagen and muscle the thickness of the aortic wall is reduced approximately 50% during an exposure to formic acid of between 24 and 36 hours (Fig. 3). Prolonged exposure of the tissue to formic acid (216 hours) causes no detectable change in the elastic lamellae either morphologically or in their special staining characteristics.

Control studies upon Achilles tendon show that elastic tissue is extremely scanty, occurring only in the sheaths surrounding bundles of collagenous fibers. Exposure of the tendon to formic acid at 45 C for six hours results in a globular hyalin swelling of the tissue with shortening and a considerable loss of special staining qualities. The tendon, except for a tiny fraction of material from the sheath, completely dissolves after an exposure to the acid of 18 hours.

Fig. 8.—High power of Figure 7 to show that reproduction of the same basic arrangement of elastic tissue descends in scale to the limits of the light microscope. Gomori's aldehyde-fuchsin; reduced 40% from mag. × 650.

Fig. 9.—Blended suspension of purified elastica neutralized with buffer on the slide. The plates have become contracted and more homogeneous, and the finer fibrillar detail is obscured. The fenestrations are smaller, fewer, and more rounded.

The blended uncentrifuged suspension prepared for electron microscopy yields interesting morphological detail when examined with the ordinary microscope. In films dried of formic acid and stained with Gomori's aldehyde-fuchsin stain there can be seen innumerable sharply contoured plates which represent fractured portions of the lamellae (Figs. 7 and 8). Within each plate occur oval fenestrations of variable size which are separated and delineated by branching anastomotic broad bands of elastic tissue. Within the thinnest bands can be seen a similar arrangement of smaller bands and fibers anastomosing about smaller and more elongate fenestrations to the limits of the light microscope. Many of the larger fenestrations are traversed by anastomosing branching fibers with resultant subloculation. These fibers fan outward at each end, unmasking finer, meshed fibrils which merge into the margins of the fenestrations. The plates vary in thickness and appear to be composed of fused extremely fine laminae. The thinnest plates have relatively large fenestrations delineated by bands of elastic tissue having a distinct inner structure. Thicker plates appear to result from fusion of the finer laminae, particularly when the long axes of the fibrillary component of the latter run at variant angles. They have smaller rounder fenestra-

Fig. 10.—Elastic lamella fully extended with formic acid after an exposure to the acid of 300 hours. The bands have spread, revealing smaller bands and fibers anastomosing about smaller fenestrations to the limits of the light mircoscope. Gomori's aldehyde-fuchsin; reduced 40% from mag. × 500.

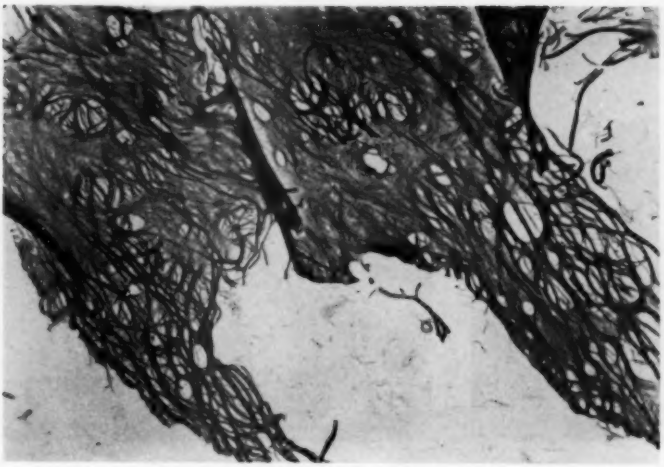

Fig. 11.—Same as Figure 10, but tissue has been three-quarters neutralized on the slide to demonstrate coacervation of the fibrillar elements and elimination of the finer fenestrations to form a more homogeneous membranous matrix in which occur relatively few rounded discrete fenestrations.

tions separated by broader, denser, and more homogeneous bands of elastic tissue. In addition to the plates the crude suspension contains frayed bands and fibers which appear to have been torn from the edges of the plates. Numerous side-branches with frayed ends project from the surfaces of these, and a fine fibrillar meshwork is seen within them, particularly at their splayed ends.

Neutralization causes shrinkage and increased density of the plates. The margins curl and become even more sharply defined (Fig. 9). The largest fenestrations become smaller, rounder, and more distinct. Subloculation within them disappears. The finer fenestrations become more elongate and eventually disappear. The elastic bands become denser, rounder, and more homogeneous, with loss of visible inner structure.

Prolonged exposure of the elastic tissue to formic acid causes progressive subdivisions into progressively finer anastomotic bands, and fibers anastamosing about progressively finer fenestrations can be detected (Fig. 10). Neutralization of the acid at any stage of exposure results in contraction of the extended networks to the original picture of neutral elastic tissue but with loss of staining density (Fig. 11).

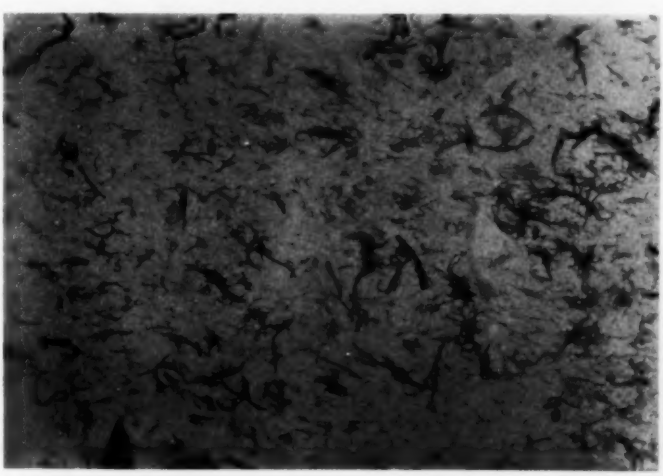

Fig. 12.—Centrifuged supernate of blended suspension of purified elastica fully extended by formic acid. The inner meshed fibrillar structure within the fibers, particularly at their expanded ends and the ends of side-branches, is clearly seen. Gomori's aldehyde-fuchsin; reduced 40% from mag. × 330.

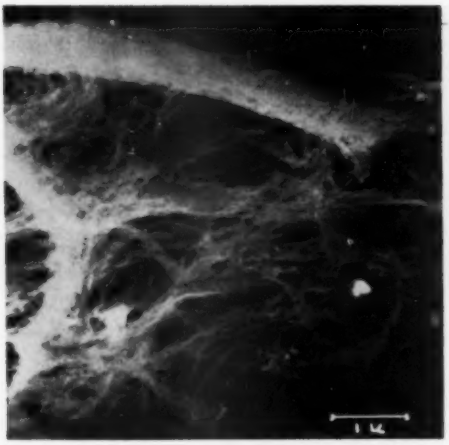

Fig. 13.—Electron micrograph of the material in Figure 12, fully extended by formic acid. The arrangement of interconnected bands anastomosing about fenestrations descends in scale toward molecular proportions. Phosphotungstic acid, chromium-shadowed.

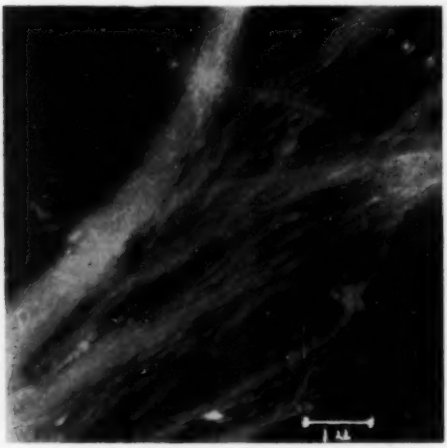

Fig. 14.—Electron micrograph of material in Figure 12, fully neutralized with buffer on the screen. The aggregates have condensed to more rounded bundles with a braided appearance and loss of finest fenestrations. Phosphotungstic acid, chromium-shadowed.

Stained films of the centrifuged supernate dried from formic acid are seen with the ordinary microscope to be composed almost entirely of the fibrillar elements torn from the margins of the plates (Fig. 12). A distinct elongated fenestrated meshwork of fibrils can be seen within these, particularly at their ends and torn side-branches. Neutralization of this material causes the inner structure and fraying to disappear resulting in discrete dense homogeneous fibers.

When dried films of the centrifuged supernatant suspension in formic acid are viewed with the electron microscope it is seen that the same structure of anastomotic branching flat bands of elastic tissue surrounding fenestrations occurs on an ever-decreasing scale to the limits of the electron microscope (Fig. 13). Neutralization of the suspension on the screens shows contraction of the flat meshworks to form more rounded ill-defined fibers composed of bundles of finer fibrils arranged much like braided rope (Fig. 14). No evidence of period is seen either in the neutralized fibers or in those extended by formic acid despite the stretching caused by rupture of the supporting collodion film with the electron beam. Also no definite unit of fiber size is seen within the range of resolution of the electron microscope.

Comment

That the aortic elastic tissue is arranged as interconnected concentric fenestrated membranes or lamellae is not a new concept but should be reemphasized because morphologists seldom view tangential sections of aorta.[11,31,52] Formic acid, by removing the compressive restraint of other materials in the aortic wall, allows relaxation of the elastic fibrillar laceworks, thus affording visualization of an inner structure. That lamellae have a finer laminar structure is suggested by the illustrations of Smith et al.[70] and Churchill et al.[16] In the present study it appears that fenestrated lamellae are composed of multiple fused finer laminae, some running with long axes in different directions without order. In the tangential plane broad bands of elastic tissue split and rejoin about fenestrations in an anastomotic manner. In the same areas lateral anastomotic interconnections with neighboring bands occur. This splitting and rejoining of portions of the bands also occurs in the third dimension, resulting in a tight

fusion of multiple laminae to form a lamella. The latter process, modified by the presence of collagen and muscle, apparently also occurs between concentrically contiguous lamellae. The result is a vast laminar syncitium with condensations at regular concentric intervals to form definitive lamellae. It is assumed that interconnecting membranes between definitive lamellae are modified by broadening of the fenestrations so that collagen and smooth muscle can pass through them. This is supported by finding portions of lamellae in which the fenestrations are considerably broader and the bands narrower and more fibrillar than in the majority.

That an inner fibrillar structure occurs within the fibers of elastic tissue has been shown by Wolpers,[76] Gross,[23] Lansing et al.[47] Schwarz and Dettmer,[68] Hall et al.,[31] and Keech et al.[37] With the extension caused by formic acid this structure can be more clearly visualized. Within larger anastomotic bands of elastic tissue, particularly in their branching expanded portions, can be seen narrower bands and fibers anastomosing about smaller fenestrations to the limits of the electron microscope. Neutralization of the acid causes recontraction of the fibrillar meshworks, with obliteration of smaller fenestrations and restoration of the original histological picture of broad dense homogeneous or longitudinally striated bands of elastic tissue surrounding relatively few distinct rounded fenestrations.

2. Determination of the Rate of Loss of Weight

Methods

Eighteen rings from 12 canine aortae are frozen (gradually to prevent cracking) and then desiccated to constant dry weight by modified lyophilization at a negative pressure of 10^{-6} mm. of mercury (96 hours). After they are weighed, the rings are immersed separately in 88% formic acid at 45 C for six hours in the manner already described. This is followed by repeated washing with formic acid and then with distilled water to neutrality. The process of freezing, desiccation, and weighing to constant weight is then repeated. Alternate exposure of the rings to formic acid and recording of the changes in their weight are carried out at regular intervals of time until the time of complete solution of the tissue. The results are plotted as the mean residual weight of the aortic rings, expressed as percentage of the original weight, remaining after increasing time of exposure to 88% formic acid at 45 C.

To test the effects of altering the concentration of formic acid, altering the temperature of 88% formic acid, and previous denaturation of the aorta, 18 rings are treated in essentially the same manner but with the following variations in technique.

A. Two rings each are tested with use of concentrations of formic acid of 22%, 44%, and 98% instead of the standard concentration of 88%.

B. Two rings each are tested with use of 88% formic acid at 4 C, 22 C, 70 C, and 100 C.

C. Two rings are fixed with 10% neutral formalin for 48 hours, and two rings are treated successively with alcohol and ether and dried at 110 C, according to the method of Hass,[24] before exposure of all to 88% formic acid at 45 C.

For purposes of control, the rate of solution of collagenous tissue by 88% formic acid at 45 C is determined by measuring the loss of dry weight of 12 strips of Achilles tendon obtained from the same 12 dogs from which the aortic rings are derived. The results are plotted in the same way as for aortic tissue. Specimens of canine smooth muscle and striated muscle and fat are exposed to formic acid and examined qualitatively at periodic intervals of time of one hour.

Results

Curves representing the loss of dry weight of aortic rings during their exposure to 88% formic acid at 45 C indicate a very

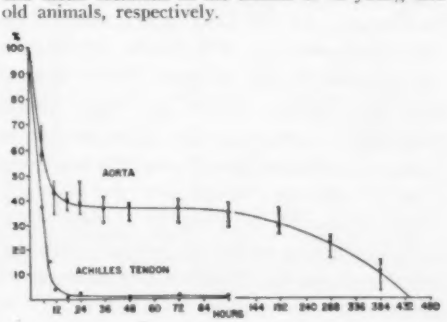

Fig. 15.—Comparison of the loss of weight of canine aorta and Achilles tendon during prolonged exposure to formic acid. The abrupt change in the curve representing loss of weight of aorta corresponds with the solution of collagen in Achilles tendon. Maxima and minima correspond in general, with rings containing less and more collagen, respectively, as in rings from proximal and distal locations in the animal or in young and old animals, respectively.

rapid loss during the first 36 hours (Fig. 15). This amounts to a mean loss of 62.9%, corresponding with a residual aortic weight of 37.1%. Maximal and minimal residual weights of 41.5% and 30.8% occur in rings having original positions in the animal of proximal and distal, respectively. After the rapid loss in weight during the initial 36 hours (extractive phase), the loss abruptly bcomes gradual and nearly constant until the time of complete solution of the tissue, between 396 and 504 hours, or a mean of 436 hours. The average loss in a 24-hour period after the initial exposure of 36 hours is 2.2%. The time of complete solution of different rings from the same animal is identical, regardless of original location, relative amount of elastic tissue, or size. Thus the original configuration of a complete aorta, including the fine intercostal arteries, persists until the moment of solution, at which time all portions disappear simultaneously (Fig. 18). On the other hand, distinct variations in the curves representing the rate of loss of weight are found among different animals and appear to correspond with their age—older animals having aortae showing increased resistance to solubility in formic acid.

Decreasing the concentration of formic acid or the temperature of 88% formic acid in the procedure results in progressive prolongation of both the extractive and the solubilizing phases, with a decreased definition between them. Increasing the temperature of 88% formic acid causes an increased rate of both extractive and solubilizing phases, but again the definition between them is obscure. At 100 C the aortic tissue completely dissolves in 88% formic acid during an exposure of 165 minutes. Previous fixation by formalin makes the tissue almost completely resistant to the action of formic acid, a loss of weight of only 8% occurring after an exposure to the acid of 144 hours. Pretreatment of the tissue by alcohol, ether, and desiccation of 110 C prolongs the extractive phase to 72 hours (an increase of 100%).

Achilles tendon treated by 88% formic acid at 45 C rapidly dissolves proportionally to the time of exposure to the acid until it is almost completely gone at 15 to 18 hours (Fig. 15). A residuum of 1.8% fails to dissolve within a period of 300 hours and is presumed to represent elastic tissue in the sheath structure of the tendon.

Uterine smooth muscle and striated muscle of the hamstring group rapidly dissolve in 88% formic acid at 45 C within a period of a few hours, leaving behind a honeycomb framework believed to represent elastic tissue in the sheath structure of these muscles. Depot fat rapidly dissolves in 88% formic acid at 45 C, resulting in a cloudy solution with a minimum of oil droplets on the surface.

Comment

The rapid loss of weight of aorta during the initial 36 hours of its exposure to 88% formic acid at 45 C closely corresponds with the complete loss of weight of collagen in Achilles tendon and the histological disappearance of all elements except elastic tissue from the aortic wall. The use of formic acid in the purification of elastic tissue depends upon the differential rate of solubility of collagen, muscle, fat, and cells on the one hand and elastic tissue on the other. That the latter also dissolves cannot be denied, the amount lost in the initial 36 hours by analysis of the curve being 3.1% of the original aortic weight. Prolonged exposure of aortic tissue to formic acid after the initial 36 hours causes an average loss of 2.2% of the original aortic weight, or 5.96% of the weight of the purified elastica within each 24-hour period until the time of complete solution at a mean exposure of 436 hours.

The average amount of elastic tissue in the canine aorta by this method is 37.1%. This compares favorably with the results obtained by other authors: Hass, 37.9% [34]; Kraemer and Miller, 34.8% [41]; Lansing et al., 43.3% [44-46]; Myers and Lang, 37.4% [57]; Lowry et al., 30% [50]; Stein and Miller, 38% [71]; Neuman and Logan, 39%.[50] It

should be emphasized, however, that there is a proportionally greater amount of elastic tissue in proximal portions of aorta than in distal portions.

Preliminary fixation of the aortic tissue by formaldehyde makes the collagen almost completely resistant to solution by formic acid. Preliminary treatment of aorta by alcohol and ether followed by air-drying at 110 C, as used by Hass,[34] decreases the rate of solution of collagen 100%, so that the optimum time of extraction becomes 72 hours instead of 36 hours. This resolves the discrepancy, noted by him, between the extractive time as indicated by the curve of loss of weight (using preliminary dehydration with alcohol, ether, and dry heat) and the extractive time as indicated by the force-extension curves (using no preliminary treatment). In the present experiments the indicated time of extraction by both tests is 36 hours.

Increasing the concentration and temperature of formic acid increases the rate of solution of collagen, but the best definition of the extractive point and the greatest simplicity of technique are obtained with use of standard concentrated formic acid (88%) at 45 C, as advocated by Hass.[34]

In comparison with other methods for obtaining pure elastin, formic acid is the only one in which morphological integrity of the elastic structure is maintained until immediately before solution of the whole tissue. Prolonged exposure to boiling water on boiling 1% acetic acid results in gradual shrinkage of the tissue, which becomes brittle and brown-black. Prolonged exposure of the tissue to boiling dilute alkali, boiling 40% urea, or 0.25 M oxalic acid results in its disintegration long before the time of complete solution.

3. Measurement of Mechanical Elasticity

Methods

The circumferences of 72 aortic rings immersed in McIlvaine's buffer (pH 5.6; μ 0.15) are measured at their midthickness by optically enlarged tracings. The effect of force to strain and the amount of subsequent recoil are measured by a kymographic modification of the method of Hass.[84] Each of the rings is slung by specially designed glass hooks between the lever of a kymograph and a fixed dependent point. Prior to this transverse grooves of the same diameter as the glass hooks are indented from the intima into the walls of the rings at opposite poles, with use of the expected maximal breaking weight of 600 gm. and exercise of due care to prevent any stretching. The rings are suspended with the hooks accurately fitting the grooves of the same diameter as the glass hooks are to immerse the tissue. The beaker in turn is surrounded by a constant water bath maintained at 25 C. Barely sufficient weight is applied to the lever of the kymogram to cause eradication of folds and irregularities in the tissue, and a base line is made. Increments of weight (5 gm. each) are applied to the lever of the kymogram to just short of the point of rupture of the rings. This point is previously determined by allowing breakage of the first four rings. With each increment of weight and after a stabilizing period of one hour, a recording is made upon the smoked drum at a mechanical magnification of five to indicate the degree of extension of the tissue. This is converted to percentage extension based upon the original circumference. The mean percentage extension of the rings for each increment in weight is then plotted to form a force-extension curve.

The undamaged rings are then placed in 88% formic acid at 45 C. At regular intervals of time the rings are removed from the acid, washed to neutrality with water, and retested in buffer as before. Four rings are destroyed at each test in order to obtain the amount of maximal extensibility before rupture. The tests are continued until after the changes in the curves have become constant (96 hours). The mean percentage force-extension curves of the same group of four aortic rings during their increasing exposure to formic acid are plotted upon the same graph for comparison.

The elastic recoil from a standard mechanically induced extension of whole aorta is demonstrated in an additional group of 36 rings with use of the same technique. After measurement and production of a base line on the smoked drum with the tissue in buffer at 25 C, four rings are mechanically stretched 80% (slightly less than the amount of maximal extensibility of purified elastica) and a recording is made. All weight, including the tare weight, is then removed for 12 hours by raising the pointer of the lever. The tare weight is then reapplied, and six hours later a recording is made. The percentage recoil is calculated upon the difference in circumference between the original and the 80% stretched tissue. The remaining rings are then placed in 88% formic acid at 45 C for regular intervals of time, are washed, and are retested in buffer as before. The mean percentage elastic

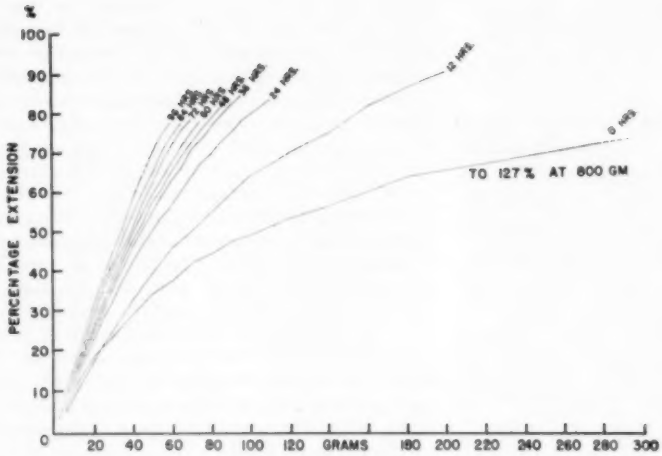

Fig. 16.—Variation in reversible mechanical extension of aorta during prolonged exposure to 88% formic acid at 45 C. Marked but decreasing changes occur in force-extension curves up to an exposure of the tissue to formic acid of 36 hours. Thereafter the changes are gradual and constant.

recoil of the rings from a mechanically extensive strain of 80% is plotted against the time of their exposure to formic acid.

No attempt is made to determine the mechanical extensibility of Achilles tendon, which is qualitatively extremely small.[9,35]

Results

The effect of time of exposure of aortic tissue to 88% formic acid at 45 C is reflected in the change in character of the serial curves of force-extension (Fig. 16). Marked but decreasing changes are evident in the curves obtained during the first 36 hours. Thereafter the changes are minimal and constant. First, the curves became progressively more steeply inclined, indicating an increase in the ratio of strain to force and greater reversible deformability or elasticity in the common usage but a decrease in Young's modulus. Second, the flat convex curve of the control tissue is gradually replaced by a near linear curve at an exposure to formic acid of 36 hours and thereafter. A slight flattening of the curves without evidence of yield[62,69] occurs immediately before rupture in all rings exposed to formic acid for 36 hours and longer.

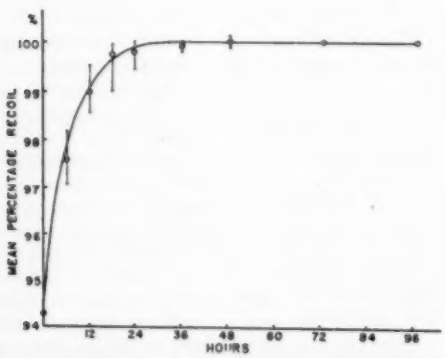

Fig. 17.—Increase of recoil of neutral aorta from a mechanically induced extension of 80% during prolonged exposure to 88% formic acid at 45 C. Complete elastic recoil occurs at an exposure of the tissue to formic acid of 36 hours and longer.

The maximal mechanical extension before rupture of the rings rapidly decreases from a mean of 127% in whole aorta to a mean of 87% in aorta exposed to formic acid for 36 hours.

The amount of elastic recoil of aortic tissue from a standard mechanical extension of 80% increases rapidly from 94.3% for whole aorta to 100% for purified elastica during the first 36 hours of exposure to formic acid (Fig. 17). Thereafter, despite a slight constant increase in hysteresis, the recoil remains complete. It should be emphasized that complete recoil occurs only after previous removal of all force, including the tare weight, before reapplying the latter and making the measurement.

Comment

The preparation of serial force-extension curves and the curve representing the in-

crease in elastic recoil of aortic rings during their exposure to 88% formic acid at 45 C for increasing periods of time is made for the sole purpose of demonstrating the removal of elements other than elastic tissue, chiefly collagen, from the aortic wall. Force is not converted to stress because of the difficulty of obtaining a suitably accurate cross-sectional area of the meshed elements of elastin (Fig. 6). Both curves indicate rapid changes toward complete elasticity during the initial 36 hours of exposure of the aortic tissue to formic acid. These correspond with its loss of weight and the histological disappearance of collagen, muscle, and cells.

The dual character of the force-extension curve of whole aorta, ascribed by Krafka [42] and Burton [14] to the mixture of collagen and elastic tissue in its wall, is lost early during the exposure to the acid through elimination of the collagenous component. The results are identical to those of Hass, who tested human material.[34-36] The force-extension curve of whole canine aorta is similar to the stress-strain curves of Saxton,[65] Wiggers,[74] Roy,[64] Wilens,[75] and Hallock and Benson,[32] with use of different animals and techniques.

Prolonged exposure of the tissue to formic acid after the initial extractive phase results in a constant daily increase of 5.9% in the ratio of strain to force, wth no change in the maximal extensibility. This apparent loss of Young's modulus is eliminated when one considers that the loss of weight of the elastic tissue and presumably its cross sectional area is also 5.9% per day.

The elastic recoil from a standard mechanical extension of whole aorta is excellent, amounting to 94.3%. This corresponds with a permanent elongation of the tissue of 4.6%, which compares favorably with the results of Hass,[34] Wilens,[75] Krafka,[42] and Saxton,[65] with use of different animals and different techniques. During the initial 36 hours of exposure to formic acid, while collagen, muscle, and cells are being extracted, the amount of elastic recoil increases to become perfect. During prolonged exposure of the purified elastic tissue to formic acid there is no loss of this perfect elastic recoil. However, hysteresis, or the time necessary to attain equilibration, increases with exposure to the acid.

4. Determination of Changes in Chemical Elasticity (Reversible Dimensional Changes Caused by Formic Acid)

Methods

Twelve aortic rings are cut open to form strips, individually segregated, and immersed in McIlvaine's buffer (pH 5.6; μ 0.15). They are examined as to color, transparency, and consistency. The dimensions of length, width, and thickness of the strips are measured at an optical enlargement of five times, with the tissue suspended in the buffer at zero stress and at a temperature of 25 C. The strips are then rinsed in distilled water, blotted, and immersed in 88% formic acid at 45 C for three hours, at which time rapid dimensional changes caused by the swelling of the collagen cease. Reexamination and remeasurement are performed with the strips suspended in the formic acid at 25 C. After this the strips are repeatedly washed in the buffer to neutrality, care being taken not to lower the ionic strength, which results in irreversible swelling of the altered collagen. They are then reexamined and remeasured as in the initial test. The process of alternating examination with measurement of the strips and their exposure to 88% formic acid at 45 C is repeated at regular intervals of time (3 hours initially to 72 hours terminally) until the time of their solution. The means of the percentage change from the original dimensions of the strips are plotted against the time of their exposure to 88% formic acid at 45 C, first while under the influence of the acid and then after having been neutralized by the buffer.

The relationship of the dimensional changes of aortic elastic tissue to the concentration of formic acid is demonstrated as follows: One ring of purified aortic elastica (aorta exposed to 88% formic acid for 36 hours and washed to neutrality) is suspended in the buffer between the lever of a kymograph and a fixed dependent point, as previously described. The half-circumference of the ring is measured directly with calipers at 25 C, and a base line is produced upon the smoked drum at a mechanical magnification of five times. The beaker of buffer is replaced by one containing distilled water at 25 C, which is changed repeatedly over a period of 12 hours. During this time the initial swelling of the tissue due to osmosis disappears and the original base line is reattained.

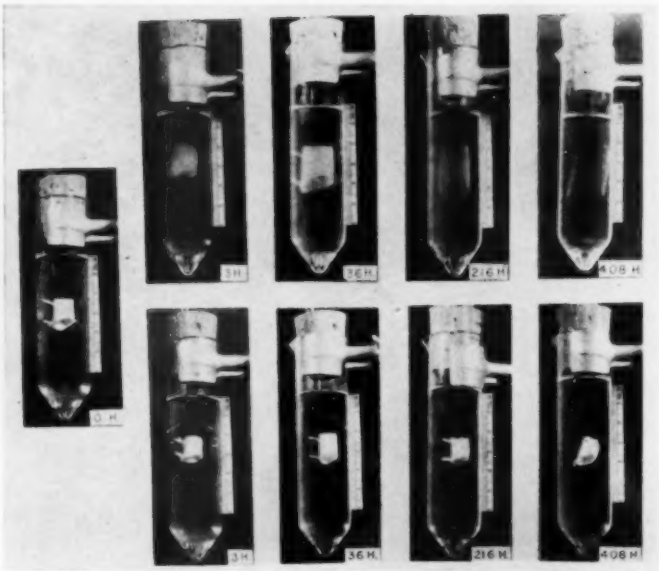

Fig. 18.—Progressive swelling of the same segment of aorta (top row) occurs during prolonged exposure to 88% formic acid at 45 C. Neutralization of the acid causes loss of the swelling seen in the top row and complete reversion (bottom row) to the original dimensions of length and circumference. The initial configuration, including the intercostal arteries, is maintained until the time of complete solution.

Beakers containing formic acid in exponentially increasing concentrations between 10^{-4}% and 1.0% and in arithmetically increasing concentrations between 0% and 98% are then progressively raised to immerse the tissue. Four changes of each solution are made for equilibration before recording upon the drum. Nitrogen is bubbled through the lowest concentrations of the acid and distilled water. The effect of the entire series of graded concentrations of formic acid is then tested in the reverse order so that the tissue is finally resuspended in distilled water. After completion of the test the rings are immersed in 88% formic acid at 45 C for 96 hours and then removed, washed to neutrality, and tested as before. The process of alternate exposure of the ring to 88% formic acid and testing is repeated until the tissue is nearly dissolved. Serial curves obtained during prolonged exposure of the ring to 88% formic acid at 45 C are plotted on the same graph. Each curve represents the percentage dimensional changes of the ring caused by varying concentrations of formic acid.

For purposes of control six strips of Achilles tendon from different dogs are used to test the effect of 88% formic acid at 45 C upon collagenous tissue. These are measured accurately in length and diameter while immersed in McIlvaine's buffer (pH 5.6; μ 0.15). They are then exposed to formic acid at 25 C for 30 minutes and again measured, first in the acid at 25 C and then after neutralization by repeated washing in the buffer at 25 C. Care is taken not to wash with water because this lowers the ionic strength and causes irreversible swelling of the tissue. Alternate measurement of the strips and their exposure to the acid for periods of 30 minutes each are accomplished until lack of coherence and transparency of the tissue makes further study impractical (six to nine hours). Attempts to correlate the dimensional changes of tendon with the concentration of formic acid fail because of the extremely sluggish response (six weeks) and the unusual swelling in girth (over 1000%) of collagenous tissue when exposed to very dilute solutions of the acid.

Results

During the initial period of three hours, 88% formic acid at 45 C causes a mean shrinkage of 3% in length and width and a mean swelling in thickness of 28% of the aortic strips (Figs. 18 to 20). Maximal contraction in length and width of 9% and swelling in thickness of 41% occur in segments from distal locations in the aorta. Minimal shrinkage in length and width (actually an extension of 4%) and minimal swelling in thickness of 17% occur in strips from proximal aortic positions. The tissue becomes hard, translucent, yellow, and rubbery. The strips tend to lose their natural curve, and those from originally distal locations become inverted, owing to the

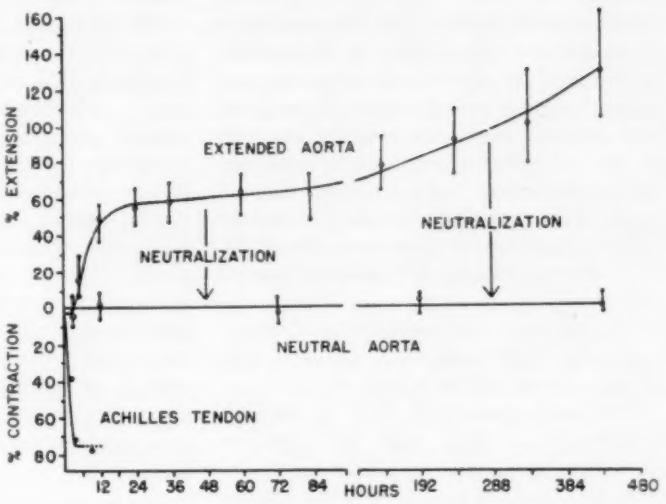

Fig. 19.—Graphic demonstration of Figure 18. Reversible chemical extension in length and width of aortic strips and contraction in length of tendon caused by prolonged exposure to formic acid. Rapid dimensional changes occur during the first 36 hours, while collagen is being dissolved. Thereafter the changes are gradual and constant. The curves of Figures 19 and 20, after the initial 36 hours, become identical if the permanently reduced thickness of purified elastica is used for calculations instead of the original aortic thickness. The extension of elastic tissue in all dimensions contrasts with the shrinkage in length and swelling in girth of tendon during exposure to formic acid.

shrinkage of the greater amount of collagen in their outer walls.

Within the next 33 hours of exposure to the acid there occurs a rapid extension in length and width of the strips to a mean increase of 59%. Maxima and minima of 70% and 47% extension are found but do not correlate with the original location of the strips in the aorta. In the same period the swelling in thickness rapidly decreases to a mean figure of 3% less than the original dimension. A maximal figure of 5.5% swelling and a minimal figure of 9% shrinkage in thickness can be correlated with original proximal and distal aortic locations, respectively.

Neutralization of the chemically extended tissue after the first 12 hours of exposure to the acid causes complete return to the original dimensions of length and width of

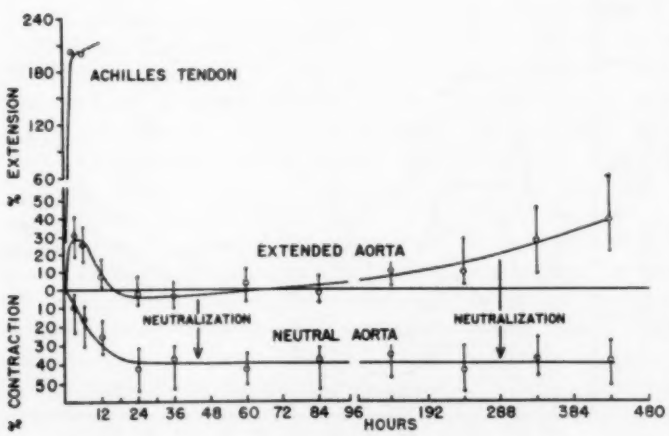

Fig. 20.—Graphic demonstration of Figure 18. Dimensional changes in thickness of aorta and girth of tendon during prolonged exposure to formic acid. Rapid dimensional changes occur during the first 36 hours, while collagen is being dissolved. Thereafter the changes are gradual and constant. The curves of Figures 19 and 20, after the initial 36 hours, become identical if the permanently reduced thickness of purified elastica is used for calculations instead of the original aortic thickness. The extension of elastic tissue in all dimensions contrasts with the shrinkage in length and swelling in girth of tendon during exposure to formic acid.

whole aorta. The neutralized thickness shows a rapid constant decrease, reaching a mean maximal loss of 39% at an exposure to the acid of 36 hours. Maximal and minimal figures of the latter correspond with originally distal and proximal locations of the rings, respectively. Thus distal segments, containing more collagen, show a greater loss of neutral thickness amounting to 53%, while proximal segments, containing less collagen, lose only 29% of their original thickness.

The chemically extended tissue at an exposure to formic acid of 36 hours is soft, elastic, opaque, and yellowish-white.

Prolonged exposure of the tissue to 88% formic acid at 45 C after the extractive phase, or initial exposure of 36 hours, results in a gradual nearly constant increase in all of its dimensions until immediately before its solution at a mean exposure of 432 hours. The final mean dimensions of length and width are 124% greater than those of whole aorta. The final mean thickness is 37% greater than the original aortic thickness. The dissimilarity of these figures is due to the fact that the percentage changes are based upon the dimensions of whole aorta and not upon those of purified elastic (aorta exposed to formic acid for 36 hours). Whereas the neutral length and width of a strip of purified elastica are identical to those of the original whole aorta from which it is derived, the neutral thickness of purified elastica is 39% less than the neutral thickness of whole aorta, owing to the loss of collagen and muscle. When the final mean percentage chemical extension of the thickness is calculated upon the thickness of the neutral elastica instead of aorta it becomes identical to those of length and width, or 124%. The percentage increment in the chemical extensibility of pure elastica in all dimensions during the time of its solution (16.5 days) thus becomes 124 (final percentage extension) − 59 (initial percentage extension) = 65 ÷ 59 = 110.1%. This amounts to a mean increment of 6.67 per 24-hour period.

At any time of exposure to 88% formic acid at 45 C after the initial 36 hours, neutralization of the acid causes the extended tissue to return to the original dimensions of length, width, and thickness of the pure elastica.

The chemically extended tissue during the period of prolonged exposure to formic acid becomes progressively more tenuous, translucent, and yellow. The neutral tissue shows only a slight loss of opacity and increased softness. The original configuration

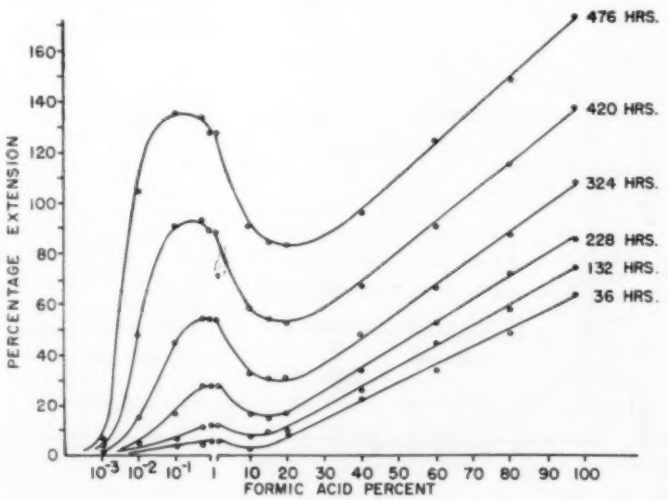

Fig. 21.—Reversible extension of purified elastica with concentration of formic acid and the effect upon it of prolonged exposure to concentrated formic acid. Two distinct curves of swelling occur in purified elastica immersed in low and high concentrations of formic acid. These increase in magnitude at a constant but unequal rate during prolonged exposure of the tissue to concentrated formic acid.

of the aorta is retained until the moment of complete solution of the tissue (Fig. 18).

Curves representing the effect of varying concentrations of formic acid upon a ring of pure elastica indicate two components of extension separated by an interval of relative contraction (Fig. 21). In a ring exposed to formic acid for 36 hours the primary response occurs at concentrations of formic acid between 0.001% and 1.0% (pH 3.9 and pH 2.2). It consists of an upward convex curve with a maximal extension of 5%, occurring at a concentration of formic acid of 1%. This is followed by a concave curve of relative contraction to a minimum figure of 3% extension at a concentration of formic acid of 10%. The secondary component of extension emerges from the curve of relative contraction, displaying a linear relationship to the concentration of formic acid between 18% and 98%. The maximal extension of 62% occurs at a concentration of formic acid of 98%. The extension of 56%, corresponding to a concentration of acid of 88%, falls within the limits of the points representing the mean extension of aortic strips exposed to 88% formic acid at 45 C for 36 hours (Fig. 19).

Prolonged exposure of the rings to 88% formic acid at 45 C causes an increase in both types of extension, the primary response increasing proportionately greater than the secondary response. The maximum of the primary extension rises from 5% at an exposure of the tissue to the acid of 36 hours to 137% at an exposure of 476 hours. During the exposure it occurs at gradually decreasing concentrations of formic acid, from 1% at an exposure of 36 hours to 0.1% at an exposure of 476 hours. The recontractive component of the primary response in this maintains a ratio of 1:1.6 with the maximum and gradually but increasingly encroaches upon the curve of the secondary response. The maxima of the latter run parallel to the curve, representing the mean extension of aortic strips (Fig. 19) but at a higher level, owing to the use of 98% instead of 88% formic acid and because the ring used for study showed unusual extensibility. The range of the secondary or linear response is gradually reduced, the initial points occurring at progressively increasing concentrations of formic acid—between 18% at an exposure of 36 hours and 37% at an exposure of 476 hours. Both types of response, regardless of the time of exposure of the tissue to 88% formic acid at 45 C, display complete reversibility.

Fresh Achilles tendon when treated with 88% formic acid at 45 C for purposes of control undergoes within the initial three hours a contraction of 78% and an increase in girth of 200% when measured at 25 C. Washing the tendon with buffer results in a 5% reextension to a neutral contraction in length of 73% and no change in the girth. Washing in water causes a tremendous irreversible swelling in girth of the tissue of over 1000%.

After the initial exposure of three hours the tendon in formic acid shows less marked dimensional changes, but the tissue becomes so ill-defined and gelatinous that further measurement is of no value.

Comment

The results of these experiments indicate that formic acid causes an irreversible contraction in length and swelling in girth of collagenous tissue, whereas it causes a reversible swelling in all dimensions of purified elastic tissue. The amount of dimensional change caused in the length of tendon is identical, as we shall see, with that caused by heat. It is found to occur at 0 C and is probably the result of lowered critical thermal shrinkage temperature caused by decreased pH, as described by Lennox [48] and Lloyd and Garrod.[49] In whole aorta the effect of formic acid is obscured by the conflicting action of the collagen and elastic tissue. Considerable variation in response is found between aorta with a relatively high content of elastin, as in proximal segments or tissue from young animals, and aorta with a relatively high content of col-

lagen, as in distal segments or segments of tissue from older animals.

After the initial exposure to 88% formic acid at 45 C for 36 hours the collagenous response is lost and the completely reversible extension in all dimensions of the pure elastica becomes clear. There is hereby confirmation for the correct time of extraction, as demonstrated by histological evaluation, changes in loss of weight, and changes in the force-extension curves of similar aortic tissue.

The chemical extension or swelling of the purified elastica increases at a constant rate during prolonged exposure to concentrated formic acid until just before solution of the tissue, when a maximum swelling of 124% is attained. This is comparable to the amount of 127% maximal mechanical extension before rupture of whole aortic rings. The rate of increase in this ability to swell in a 24-hour period is 6.67%, which, compared to the rate of loss of weight of 5.96%, yields a ratio of 1.12:1.

The action of concentrated formic acid in causing extension of the elastic meshworks is not clear at the present time. That it is not strictly an effect of pH is obvious when curves representing the swelling of pure elastica caused by varying concentrations of formic acid are analyzed. True, the initial, or primary, extensive response is the result of increasing hydrogen-ion concentration and can be reproduced using any acid of the same pH and ionic strength. This response increases as a geometric progression with prolonged exposure of the tissue to concentrated formic acid at 45 C and is assumed to be caused by an increase of exposed reactive groups in the tissue, with increased binding of hydrogen ions and concomitant hydration. The recontractive phase of the response is undoubtedly due to the effect of the greater ionic strength in the higher concentrations of the acids, which counteracts the hydrogen-ion effect. Indeed, as the concentration of acids other than formic acid is increased the tissue shows progressive shrinkage. This recontractive phase can be reproduced by adding to dilute concentrations of acid which cause maximal swelling of the tissue sodium chloride in amounts calculated to yield the same ionic strength as found in higher concentrations of acid which cause shrinkage of the tissue. It is not reproduced by adding glucose in a similar manner.

In contrast to other acids, formic acid in higher concentrations causes a distinct and separate extensive response in elastic tissue. This is reversible and is proportional to the concentration of formic acid between 18% and 98%. Its magnitude gradually increases at a constant rate during prolonged exposure to 88% formic acid at 45 C until the time of solution of the tissue. A mechanism whereby concentrated formic acid causes this extension is suggested as follows. Elastic tissue occurs as a meshwork of branching bands, fibers, and fibrils aggregated in such a manner that larger elements are composed of and interconnected by smaller elements on a progressively smaller scale to the limits of resolution of the electron microscope. Concentrated formic acid causes the finest meshworks to undergo immediate maximal extension, with subsequent complete dispersion and formation of molecular solution. They are replaced by exposure of similar meshworks within the next larger order of aggregation, which have undergone a more limited extension due to their more massive anastomic interconnections. This process is repeated for meshworks of progressively increasing dimensions and firmness of cohesive aggregational binding. The maximal initial chemical extension of the whole tissue is governed by the relatively limited extension of the firmly bound largest aggregates. During prolonged exposure to formic acid the finest elements dissolve, with weakening of interconnections between elements of larger aggregates. The latter can now extend more fully, exposing more of the finest elements in the process. These undergo maximal extension and solution, and the entire process is repeated. Thus at one end of the scale there

is a constant process of maximal extension and solution of the finest elements, while at the other end there occurs a limited but constantly increasing extension of the largest and hence more firmly bound aggregated elements which govern the dimensions of the whole tissue. Immediately before solution these largest elements are mere ghosts of their former structure, being composed of barely visible and submicroscopic aggregated fibrillar meshworks, all displaying near-maximal and maximal chemical extension. The phenomenon of the abrupt disappearance of the original configuration of aorta coinciding with the moment of complete solution of all tissue within it is thus explained (Fig. 18). Since the rates of solution and chemical extension of elastica are interdependent, they should have a definite relationship. This is found to be a ratio of 1.12:1.00.

5. Determination of Changes in Thermal Elasticity (Reversible Dimensional Changes Caused by Changes in Temperature)

Methods

Twenty-four aortic rings are opened into strips in order to study the effect of 88% formic acid at 45 C upon the thermal response of aortic tissue. A modification of the method described by McCartney is used.[60] The strip to be tested is suspended between the lever of the kymograph and a fixed dependent hook within a vessel containing McIlvaine's buffer (pH 5.6; μ 0.15). Into the vessel are placed a stirring device and thermometer. The tension on the strip is adjusted to barely eradicate folds and wrinkles in the tissue. The temperature of the strip is reduced to 0 C by means of an ice-saline bath surrounding the vessel, and the length is measured directly by means of calipers. After a base line has been made upon the smoked drum, the temperature is gradually raised to 100 C and lowered again to 0 C in increments of 5 C. Sufficient time (five minutes) is allowed after each change in temperature to allow stabilization before a recording is made upon the drum. Measurements from the kymographic tracing are converted into percentage deviations of contraction from the original length of the strip at 0 C. The mean results of four strips of whole aorta are plotted against increasing and decreasing temperature between 0 and 100 C. The remaining 20 rings are immersed in 88% formic acid at 45 C. Four strips are removed at exposures to the acid of 3, 6, 12, 24, and 36 hours. These are washed to neutrality in the buffer, after which their thermoelastic behavior is demonstrated as before. Since no alteration of the tissue is found to result from repeated heating of strips exposed to formic acid for 36 hours and longer, after the collagen has been extracted, the last four strips are repeatedly used in a serial manner. First they are exposed to the acid for regular periods of time and then after being washed in buffer they are tested for thermoelasticity. The procedure is continued until the rings become too delicate for handling (360 hours). In this way a series of curves are obtained which demonstrate the effect upon the thermoelastic properties of aortic tissue caused by its prolonged exposure to 88% formic acid at 45 C.

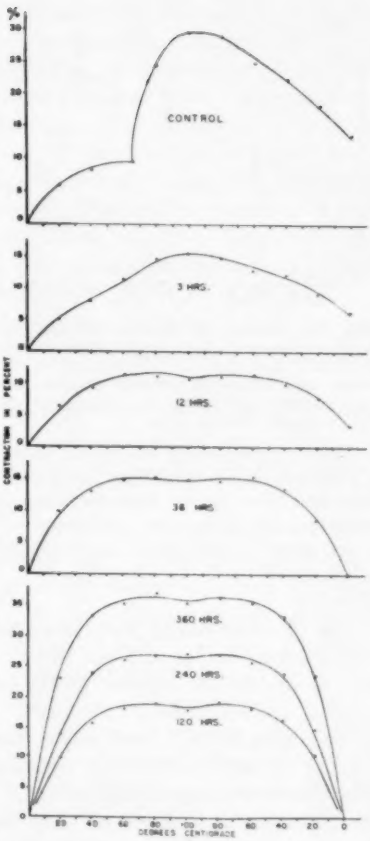

Fig. 22.—Thermal contraction and reextension of neutral aorta at intervals of time during exposure to 88% formic acid at 45 C. Two distinct curves of contraction of whole control aorta occur with increasing temperature. During the initial 36 hours of exposure of the tissue to concentrated formic acid the second curve, corresponding to the contraction of collagen, is eliminated and thermoelastic recoil becomes perfect.

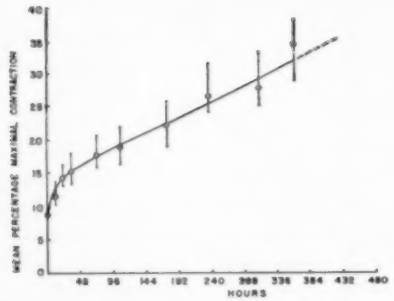

Fig. 23.—Increase in maximal thermal contraction of canine aorta during prolonged exposure to 88% formic acid at 45 C. The amount of maximal thermal contraction of purified elastica increases at a constant rate during its prolonged exposure to concentrated formic acid.

In order to demonstrate the rate of increase of the maximal thermocontractility of aortic rings during their exposure to formic acid the figures representing the mean total contraction of each test are plotted against the time of exposure of the rings to the acid.

Results

Strips of whole control aorta when heated in physiological buffer undergo two distinct forms of contraction, depicted on the kymograms as convex curves (Fig. 22). The first, beginning at 0 C, shows major changes up to 50 C, with diminishing response to a maximum shortening of 9% at 60 C. The second, starting at precisely 65 C, is superimposed on the first. It shows major changes between 65 C and 80 C, with diminishing response to a maximum shortening of 28% at 95 C. Cooling causes a nearly linear type of extension of 15% or elastic recoil of 53%. Exposure of aortic rings to concentrated formic acid at 45 C for regularly increasing periods of time before washing and thermally testing causes the following changes. The second convex curve becomes shifted into a cooler temperature range and gradually decreases in amplitude. The primary convex curve becomes more prominent, particularly in the lowest temperature range. The curve of reextension becomes convex and gradually approaches the base line. At a time of exposure to formic acid of 36 hours the rings exhibit a single symmetrical curve of thermal contraction and reextension, with major changes occurring between 0 and 45 C and a gradual flattening to a maximum shortening of 15% at 75 C. The reextension and recontraction of 1% occurring between 85 C and 100 C are unexplained but appear to be related to the vaporization of water.

Prolonged exposure of the rings to concentrated formic acid at 45 C after the initial period of 36 hours results in a constant gradual increase in the amount of their mean maximal thermocontractility (Fig. 23). This attains a shortening of 32% at 360 hours, when the tissue becomes too delicate for handling. Projection of the curve to the time of solution of adjacent rings (432 hours) indicates a maximal attainable thermocontractility of 36%. The percentage increase in thermocontractility of purified elastic tissue during the time of its solution (16.5 days) thus becomes 36 (final percentage contractility) − 15 (initial percentage contractility) = 21 ÷ 15 = 140%, or an increase of 8.48% in a 24-hour period.

Collagenous tissue of Achilles tendon, when treated similarly as a control, shows no dimensional change until the temperature of 65 C is attained, at which point contraction begins (Fig. 24). If this temperature is maintained, contraction of 78% is attained

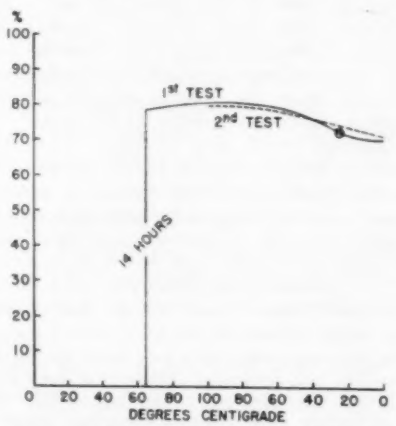

Fig. 24.—Alteration in length of Achilles tendon with temperature. Collagen of Achilles tendon shows abrupt thermal contraction at 65 C coinciding with the second thermal contraction of whole aorta. At 25 C the contraction of tendon of 73% is equal to that caused by concentrated formic acid.

in a period of 14 hours. Raising the temperature at this time results in further contraction to a maximum of 80% between 85 and 100 C. The diameter of the tendon increases 200% during the procedure. Cooling the contracted tissue in steps of 5 C results in a reverse-S-shaped curve of reextension of 10%, with major changes occurring between 65 and 20 C. At 25 C the amount of shortening is found to be 73%. Reapplication of heat and cold results in a nearly symmetrical thermoelastic curve of contraction and reextension within and slightly less than the limits of the original recoil and amounting to a maximum of 33% of the new contracted length of the heat-denatured tissue at 0 C.

Comment

The changes in the thermoelasticity of aortic tissue during exposure to 88% formic acid at 45 C add confirmation to the results of previous tests, indicating an optimal extractive time of 36 hours for obtaining pure elastic tissues from whole aorta. The secondary contraction, with imperfect recoil occurring at 65 C (the temperature at which collagen of Achilles tendon contracts), is completely eliminated at this time, leaving a single symmetrical curve of contraction and reextension.

The maximal amount of thermocontractility of purified elastica increases at a constant rate during prolonged exposure to formic acid. The daily rate of this increase is 8.48%, which, compared to the daily rate of loss of weight of 5.96%, yields a ratio of 1.42:1.0.

The thermoelasticity of elastic tissue has been described, with use of thermodynamic principles, as being similar to that of rubber, because both substances display the Gough-Joule effect.[38,49,55,56] The latter anomalous contraction of stretched elastomers with applied heat is explained by the increased thermal agitation of the extended molecular chains, which overbalances the cohesion of the relatively few cross linkages connecting them. The chains thus coil and shorten into their more probably random arrangement. That the thermoelasticity of elastic tissue as described in our experiments is not exactly the same as that of rubber is indicated by the following two facts. First, in contrast to rubber, elastic tissue does exhibit thermoelasticity without the necessity of first being stretched. Second, in contrast to rubber, elastic tissue must be fully hydrated in order to demonstrate the full range of thermoelasticity. The last statement, in contradiction to McCartney, can be proved simply by observing the contraction of the tissue as it dries in air. A similar contraction occurs when the tissue is transferred from water at 0 C to dehydrating agents, such as alcohol, acetone, glycerin, and hypertonic glucose and saline solutions also at 0 C. From this it also appears that water performs a more essential role in the thermal dimensional changes of elastic tissue than that of mere lubrication as suggested by Lloyd and Garrod.[49] If it is admitted that hydration and dehydration of the tissue are related to its thermoelasticity, it then becomes possible to explain the increase in range of its neutral thermoelastic response as it is undergoing loss of weight during prolonged exposure to concentrated warm formic acid. Since the neutral dimensions do not change in this time, the tissue must be becoming proportionately more hydrated.

The reversible contraction of elastic tissue with heat is entirely unlike the irreversible contraction of native collagen[9,25] in both character and degree. Heat-denatured collagen, however, is rubbery and has some degree of reversible thermal contractility. This differs in character and in amount from that of elastic tissue. It exhibits sluggish incomplete elastic recoil, with lack of reproductibility in serial tests. Its amount fails to increase with prolonged exposure to formic acid.

The shortening of tendon caused by heat is identical to that caused by formic acid when both are measured in buffer at 25 C, the amount of each being 73%.

Summary

Five tests are utilized to determine the time of exposure to 88% formic acid at 45 C of canine aorta required to obtain purified elastic tissue. The results of all of the tests, consisting of morphological examination, determination of loss of weight, measurement of changes in mechanical elasticity, measurement of changes in chemical elasticity, and measurement of changes in thermal elasticity, indicate an optimal extractive time of exposure of 36 hours.

In the performance of the tests insight into the structure of aortic elastic tissue on a macromolecular scale is obtained. The tissue is seen to occur as a syncytium of radially interconnected fenestrated membranes with concentric condensations at regular radial intervals to form definitive lamellae. Within the membranes, bands of elastic tissue split and anastomose about fenestrations in the tangential plane and with concentrically contiguous membranes in the radial plane. Within the homogeneous bands finer bands, fibers and fibrils anastomosing about finer fenestrations to the limits of the electron microscope can be demonstrated by extension of the elastic meshworks with formic acid. Neutralization of the latter allows reaggregation of the tissue to its original state, with disappearance of the finer subdivisions and the reappearance of the homogeneous membranous character. This reversibility is incompatible with theories that elastic tissue is composed of fibers of elastin embedded in a matrix of a more readily soluble mucopolysaccharide or lipoprotein.[18,26-31,43,45,47] It is compatible with the studies of Wood, who demonstrated that mucopolysaccharide is not essential to the integrity of elastic tissue.[77,78] It extends to a macromolecular scale the conclusions of Partridge et al. that elastin is a homogeneous material on a molecular scale.[60,61]

A reasonable explanation of the effects caused by prolonged action of formic acid upon elastic tissue is presented. This correlates the constant gradual solution of the tissue, with its constant gradual increase in chemically extended dimensions without change in neutral dimensions, with its constant gradual increase in thermocontractility, and with its morphological arrangement of larger aggregated structures being composed of similar finer aggregated structures on a progressively smaller scale to molecular proportions. The fact that the maximal mechanical extensibility of whole aorta before rupture is equal to the maximal amount of chemical extensibility before solution in formic acid is of considerable interest in view of the loss of mechanical extensibility of elastic tissue, resulting from the removal of collagen, as pointed out by Wood.[78] The marked response of the finest elements to extremely dilute concentrations of hydrogen ion, provided the ionic strength is low, may have some practical importance in the study of bodily structures containing elastic tissue.

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REFERENCES

1. Adair, G. S.; Davis, H. F., and Partridge, S. M.: A Soluble Protein Derived from Elastin, Nature, London 167:605, 1951.

2. Baló, J., and Banga, I.: Die Zerstörung der elastischen Fasern der Gefässwand, Schweiz. Ztschr. Path. u. Bakt. 12:350-362, 1949.

3. Baló, J., and Banga, I.: Elastase and Elastase-Inhibitor, Nature, London 164:491, 1949.

4. Baló, J., and Banga, I.: The Elastolytic Activity of Pancreatic Extracts, Biochem. J. 46: 384-387, 1950.

5. Banga, I.: Isolation and Crystallisation of Elastase from the Pancreas of Cattle, Acta physiol. hung. 3:317-324, 1952.

6. Banga, I.: Thermal Contraction of Collagen and Its Dissolution with Elastase, Nature, London 172:1099, 1953.

7. Banga, I., and Baló, J.: Elastin and Elastase, Nature, London 171:44, 1953.

8. Banga, I., and Schuler, D.: Contributions to the Structure of Elastin with Special Reference to the Action of Elastase, Acta physiol. hung. 4:13-24, 1953.

9. Bear, R. S.: The Structure of Collagen Fibrils, in Advances Protein Chem. 7:69-160, 1952.

10. Bendall, J. R.: The Titration Curves of Elastin and of the Derived α- and β-Proteins, Biochem. J. 61:31-32, 1955.

11. Benninghoff, H.: Über die Beziehungen zwischen elastischen Gerust und glatter Muskulatur in der Arterienwand und ihre funkionelle Bedeu-

tung, Ztschr. Zellforsch u. mikr. Anat. 6:348-396, 1928.

12. Bowen, T. J.: Physical Studies on a Soluble Protein Obtained by the Degradation of Elastin with Urea, Biochem. J. 55:766-768, 1953.

13. Bowes, J. H., and Kenten, R. H.: Some Observations on the Amino-Acid Distribution of Collagen, Elastin and Reticular Tissue from Different Sources, Biochem. J. 45:281-285, 1949.

14. Burton, A. C.: Relation of Structure to Function of the Tissues of the Wall of Blood Vessels, Physiol. Rev. 34:619-642, 1954.

15. Chittenden, R. H., and Hart, H. S.: Elastin and the Elastose Bodies, Studies, Sheffield Scientific School, Yale University Laboratory of Physiological Chemistry 3:19-38, 1887-1888.

16. Churchill, D. W.; Gelfant, S.; Lalich, J. J.; and Angevine, D. M.: Alterations in the Polysaccharides and Elastic Fibers in the Aortas of Rats Fed Toxic Lathyrus Factor, Lab. Invest. 4: 1-8, 1955.

17. Cowdry, E. V.: The Structure and Physiology of Blood Vessels in Arteriosclerosis: A Survey of the Problem, edited by E. V. Cowdry, New York, The Macmillan Company, 1933, pp. 53-76.

18. Dempsey, E. W., and Lansing, A. I.: Elastic Tissue, in International Review of Cytology, edited by G. H. Bourne and J. F. Danielli, New York, Academic Press, Inc., 1954, Vol. 3, pp. 437-453.

19. Eulenberg, quoted by Schultze, M. S.: Über die chemische Zusammensetzung der Arterienhäute: Annal. Chemie u. Pharmacie 71:277-295, 1849.

20. Felsher, Z.: Collagen, Reticulin and Elastin, in Rothman, S.: Physiology and Biochemistry of Skin, Chicago, The University of Chicago Press, 1954, pp. 391-417.

21. Florence, G., and Loiseleur, J.: Contribution à l'étude des substances collagènes: I. Préparation et propriétés de différents collagènes, Bull. Soc. chim. biol. 16:52-63, 1934.

22. Gomori, G.: Aldehyde-Fuchsin: A New Stain for Elastic Tissue, Am. J. Clin. Path. 20: 665-666, 1950.

23. Gross, J.: The Structure of Elastic Tissue as Studied with the Electron Microscope, J. Exper. Med. 89:699-708i, 1949.

24. Gross, J.: Connective Tissue Fine Structure and Some Methods for Its Analysis, J. Gerontol. 5:343-360, 1950.

25. Gustavson, K. H.: The Chemistry and Reactivity of Collagen, New York, Academic Press, Inc., 1956, pp. 211-227.

26. Hall, D. A.: Elastin from Human Tissue and from Ox Ligament, Nature, London 168:513, 1951.

27. Hall, D. A.: Studies on the Complex Nature of the Elastin-Elastase System, Biochem. J. 55: XXXV, 1953.

28. Hall, D. A.: The Reaction Between Elastase and Elastic Tissue: 1. The Substrate, Biochem. J. 59:459-465, 1955.

29. Hall, D. A., and Gardiner, J. E.: The Reaction Between Elastase and Elastic Tissue: 2. Preparation and Properties of the Enzyme, Biochem. J. 59:465-470, 1955.

30. Hall, D. A.; Reed, R., and Tunbridge, R. E.: Structure of Elastic Tissue, Nature, London 170: 264-266, 1952.

31. Hall, D. A.; Reed, R., and Tunbridge, R. E.: Electron Microscope Studies of Elastic Tissue, J. Exper. Cell Res. 8:35-48, 1955.

32. Hallock, F., and Benson, J. C.: Studies on Elastic Properties of Human Isolated Aorta, J. Clin. Invest. 16:595-602, 1937.

33. Hass, G. M.: Elastic Tissue, Arch. Path. 27: 334-65, 1939.

34. Hass, G. M.: Elastic Tissue: I. Description of a Method for the Isolation of Elastic Tissue, Arch. Path. 34:807-819, 1942.

35. Hass, G. M.: Elastic Tissue: II. A Study of the Elasticity and Tensile Strength of Elastic Tissue Isolated from the Human Aorta, Arch. Path. 34:971-981, 1942.

36. Hass, G. M.: Elastic Tissue: III. Relations Between the Structure of the Aging Aorta and the Properties of the Isolated Aortic Elastic Tissue, Arch. Path. 35:29-45, 1943.

37. Keech, M. K.; Reed, R., and Wood, M. J.: Further Observations on the Transformation of Collagen Fibrils into "Elastin": An Electron-Microscope Study, J. Path. & Bact. 71:477-493, 1956.

38. King, A. L., and Lawton, R. W.: Elasticity of Body Tissues, in Medical Physics, edited by M. O. Glasser, Chicago, The Year Book Publishers, Inc., 1950, pp. 302-316.

39. Kornhauser, S. I.: A Revised Method for the Quad Stain, Stain Technol. 20:33-35, 1945.

40. Kornhauser, S. I.: Orcein and Elastic Fibers, Stain Technol. 27:131-134, 1952.

41. Kraemer, D. M., and Miller, H.: Elastin Content of the Albuminoid Fraction of Human Aorta, A. M. A. Arch. Path. 55:70-72, 1953.

42. Krafka, J., Jr.: Changes in Elasticity of the Aorta with Age, Arch. Path. 29:303-309, 1940.

43. Lansing, A. I.: Ageing of Elastic Tissue and the Systemic Effects of Elastase, in Ciba Foundation Colloquia on Ageing: Volume I: General Aspects, edited by G. E. W. Wolstenholme and M. P. Cameron, Boston, Little, Brown & Company, 1955, pp. 88-102.

44. Lansing, A. E.; Alex, M., and Rosenthal, T. B.: Calcium and Elastin in Human Arteriosclerosis, J. Gerontol. 5:112-119, 1950.

45. Lansing, A. I.; Roberts, E.; Ramasarma, G. B.; Rosenthal, T. B., and Alex, M.: Changes with Age in Amino Acid Composition of Arterial

Elastin, Proc. Soc. Exper. Biol. & Med. 76:714-17, 1951.

46. Lansing, A. I.; Rosenthal, T. B., and Alex, M.: Significance of Medial Age Changes in the Human Pulmonary Artery, J. Gerontol. 5:211-215, 1950.

47. Lansing, A. I.; Rosenthal, T. B.; Alex, M., and Dempsey, E. W.: The Structure and Chemical Characterization of Elastic Fibers as Revealed by Elastase and by Electron Microscopy, Anat. Rec. 114:555-575, 1952.

48. Lennox, G. F.: Shrinkage of Collagen, Biochim. et biophys. acta 3:170-187, 1949.

49. Lloyd, D. J.; Jordan, D., and Garrod, M.: A Contribution to the Theory of the Structure of Protein Fibres with Special Reference to the So-Called Thermal Shrinkage of the Collagen Fibre, Tr. Faraday Soc. 64:441-451, 1948.

50. Lowry, O. H.; Gilligan, D. R., and Katersky, E. M.: The Determination of Collagen and Elastin in Tissues, with Results Obtained in Various Normal Tissues from Different Species, J. Biol. Chem. 139:795-804, 1941.

51. Loiseleur, M. J.: Sur quelques Propriétés des protéides en solution organique, Bull. Soc. chim. biol. 14:1088-1100, 1932.

52. Maximow, A. A., and Bloom, W.: A Textbook of Histology, Ed. 6, Philadelphia, W. B. Saunders Company, 1952, pp. 52, 71, 209-215.

53. McCartney, J. E.: Heat Contraction of Elastic Tissue, Quart. J. Exper. Physiol. 7:103-114, 1914.

54. McManus, J. F.: A Histological and Histochemical Use of Periodic Acid, Stain Technol. 23: 99-108, 1948.

55. Meyer, K. H.: Classification of Fibrous Proteins, Nature, London 164:34-35, 1949.

56. Meyer, K. H., and Ferri, C.: Die elastischen Eigenschaften der elastischen und kollagenen Fasern und ihre molekulare Deutung, Arch. ges. Physiol. 238:78-90, 1937.

57. Myers, V. C., and Lang, W. W.: Some Chemical Changes in the Human Thoracic Aorta Accompanying the Aging Process Method, J. Gerontol. 1:441-444, 1946.

58. Neuman, R. E.: Amino Acid Composition of Gelatins, Collagens and Elastins from Different Sources, Arch. Biochem. 24:289-298, 1949.

59. Neuman, R. E., and Logan, M. A.: Determination of Collagen and Elastin in Tissues, J. Biol. Chem. 186:549-556, 1950.

60. Partridge, S. M., and Davis, H. F.: The Chemistry of Connective Tissue: 3. Composition of the Soluble Proteins Derived from Elastin, Biochem. J. 61:21-30, 1955.

61. Partridge, S. M.; Davis, H. F., and Adair, G. S.: The Chemistry of Connective Tissue: 2. Soluble Proteins Derived from Partial Hydrolysis of Elastin, Biochem. J. 61:11-21, 1955.

62. Pryor, M. G. M.: The Rheology of Muscle, in Deformation and Flow in Biological Systems, edited by A. Frey-Wyssling, New York, Interscience Publishers, Inc., 1952, pp. 157-178.

63. Richards, A. N., and Gies, W. J.: Chemical Studies of Elastin, Mucoid and Other Proteids in Elastic Tissue with Notes on Ligament Extractives, Am. J. Physiol. 7:93-134, 1902.

64. Roy, C. S.: The Elastic Properties of the Arterial Wall, J. Physiol. 3:125-159, 1880.

65. Saxton, J. A., Jr.: Elastic Properties of the Rabbit Aorta in Relation to Age, Arch. Path. 34: 262-274, 1942.

66. Schepilewsky, E.: Über die Bestimmung des Bindegewebes im Muskel, Arch. Hyg. 34:348-360, 1899.

67. Schwarz, H.: Untersuchungen über die chemische Beschaffenheit der elastischen Substanz der Aorta, Ztschr. physiol. Chem. 18:487-512, 1894.

68. Schwarz, W., and Dettmer, N.: Elektronenmikroskopische Untersuchung des elastischen Gewebes in der Media der menschlichen Aorta, Arch. path. Anat. 323:243-268, 1953.

69. Seifriz, W.: The Rheological Properties of Protoplasm, in Deformation and Flow in Biological Systems, edited by A. Frey-Wyssling, New York, Interscience Publishers, Inc., 1952, pp. 39:47.

70. Smith, C.; Seitner, M. M., and Wang, H. P.: Aging Changes in the Tunica Media of the Aorta, Anat. Rec. 109:13-39, 1951.

71. Stein, W. H., and Miller, E. G., Jr.: The Composition of Elastin, J. Biol. Chem. 125:599-614, 1938.

72. Vandegrift, G. W., and Gies, W. J.: The Composition of Yellow Fibrous Connective Tissue, Am. J. Physiol. 5:287-297, 1901.

73. Wells, H. G.: The Chemistry of Arteriosclerosis, in Arteriosclerosis: A Survey of the Problem, edited by E. V. Cowdry, New York, The Macmillan Company, 1933, pp. 323-353.

74. Wiggers, C. J.: The Influence of Vascular Factors on Mean Pressure, Pulse Pressure and Phasic Peripheral Flow, Am. J. Physiol. 123:644-658, 1938.

75. Wilens, S. L.: The Post Mortem Elasticity of the Adult Human Aorta: Its Relation to Age and to the Distribution of Intimal Atheromas, Am. J. Path. 13:811-834, 1937.

76. Wolpers, C.: Zur electronen-mikroskopischen Darstellung elastischer Gewebeselemente, Klin. Wchnschr. 23:169-172, 1944.

77. Wood, G. C.: The Stabilization of Elastin by a Polysaccharide, Biochem. J. 55:XXXIV-XXXV, 1953.

78. Wood, G. C.: Some Tensile Properties of Elastic Tissue, Biochim. et biophys. acta 5:311-324, 1954.

Aminonucleoside Nephrosis in Rats

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Puromycin, an antibiotic derived from Streptomyces alboniger by Porter, in 1952,[1] and its related aminonucleoside (6-dimethylaminopurine-3-amino-D-ribose) have been observed to have a beneficial effect in the treatment of such parasitic infestations as amebiasis[2] and trypanomiasis[3,4] and to exhibit an inhibitory action on the growth of some Gram-positive and Gram-negative bacteria.[5] In addition, these drugs have demonstrated a retardative effect on neoplastic growth in mice.[5,6] Although toxic manifestations of these agents have not been observed in man, they have been noted to produce a nephrotic syndrome in rats.[7,8] The aminonucleoside appears more potent in this regard, with a requirement of as little as 0.003 ml. of a 0.5% aqueous solution per gram of body weight, administered subcutaneously for 8 to 12 consecutive days.

The kidneys from rats receiving toxic doses of aminonucleoside appear pale and swollen. Fiegelson and associates[8] observed numerous casts within tubular lumens as well as sudanophilic material in tubular epithelium, endothelium, and blood vessels. Significant glomerular alteration was not evident. Because of this relative paucity of renal change they considered that "profound metabolic aberrations" resulted from the administration of aminonucleoside and that such an effect was possibly responsible for the nephrotic syndrome encountered in these animals. On the other hand, Frenk and associates[7] described occasional duplication of the glomerular basement membrane as well as a diffuse thickening of this structure in sections stained by the Hale method for the demonstration of acid mucopolysaccharides. In addition, a marked loss of mitochondrial elements was evident in the epithelial cells of convoluted tubules. These latter investigators indicated that these structural alterations were reversible in short-term experiments (5 to 20 days). Collagenization or glomerular proliferation were not apparent as in the renal lesions induced by injections of anti-rat-kidney rabbit serum.

Recently, we[9] have observed a depletion of histochemically demonstrable succinic dehydrogenase and cytochrome oxidase (G-nadi oxidase) in renal tubules of rats receiving highly potent anti-rat-kidney rabbit serum. Certain considerations suggested that this alteration was related to the massive proteinuria exhibited by these animals rather than to direct tubular damage mediated by the nephrotoxic serum. Since the injection of aminonucleoside appeared to represent another modality for producing proteinuria and the nephrotic state, it was considered pertinent to explore its effect on these renal respiratory enzymes as well as to characterize further its biochemical, pathologic, and clinical features and to compare the latter with those noted in rats after the injection of anti-rat-kidney rabbit serum. Because previous studies[7,8] concerning the nephrotoxicity of aminonucleoside were performed with immature rats, it was considered worth while to ascertain whether a similar effect could be obtained in mature animals.

Method and Material

General Plan and Dosage of Aminonucleoside.—One hundred ninety-six adult female Wistar rats weighing between 150 and 200 gm. were utilized in

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this study. One hundred sixty received aminonucleoside,* and thirty-six were utilized as untreated controls. Seventy-eight rats received 0.003 ml. per gram of 0.5% aqueous solution of aminonucleoside subcutaneously for 9 to 11 consecutive days. Fifty-seven were killed by decapitation 14 days after the initial injection. Five animals died before 14 days. Sixteen rats also received 10 daily injections of 0.003 ml. of aminonucleoside per gram but were observed until they died or were killed 45 days after the initial injection. A similar number also received 0.003 ml. of aminonucleoside per gram but were killed after six injections.

Eighteen rats were given 10 consecutive daily subcutaneous injections of 0.0015 ml. of aminonucleoside per gram and were killed on the 14th day of the experiment. Ten additional rats received a similar dose but were observed for 45 days. Twenty rats received 0.006 ml. per gram subcutaneously for eight days; ten were killed on the ninth day, and ten were observed until they died.

Eight rats were given one intravenous injection of 0.006 ml. of aminonucleoside per gram, and ten received two consecutive daily intravenous injections of a similar dose. Animals receiving intravenous injections were killed 14 days after the initial injection.

All animals were placed in individual metabolism cages and allowed water and Purina Chow ad libitum except for 18 hours prior to killing. Untreated controls were killed with the experimental animals, and biochemical and staining techniques were performed simultaneously. Weights were recorded at the beginning of the experiment and at the time of killing.

Estimation of Proteinuria.—Urine was collected during an 18- to 24-hour period in clean flasks fitted into paraffin-coated funnels. Protein content was determined by the method of Looney and Walsh.[10] Previous observations indicated that small amounts of food particles occasionally present within the urine sample did not affect the result.

Biochemical Determinations.—Blood was obtained at the time of killing, and cholesterol was determined by the method of Schonheimer and Sperry; total lipid, by the method of de la Huerga, Yesinick, and Popper[11]; N. P. N., by the Kjeldahl technique, and total serum protein, by the biuret method.

Estimation of Protein Reabsorption with Evans Blue Dye.—Eight rats that received 0.003 ml. of aminonucleoside per gram for nine consecutive days were given an intravenous injection of 5 mg. of Evans blue dye on the 13th experimental day and killed 24 hours later. A similar number of animals received a similar dose of aminonucleoside but received an intravenous injection of Evans blue dye on the fifth day and were killed one day later. The urines and kidneys of rats receiving Evans blue after aminonucleoside administration were compared with those from untreated controls that received a similar dose of the dye. In addition, frozen sections were prepared from the kidneys of both groups for the detection of intracellular deposits of the dye.

Electrophoretic Studies.—Paper electrophoresis was performed on the serum and urine of 10 rats that received 0.003 ml. of aminonucleoside per gram subcutaneously for 9 consecutive days and were killed 14 days after the initial injection. The technique utilized has been described elsewhere.[12]

Histologic and Histochemical Studies.—The kidneys from all animals were quickly removed at the time of killing and longitudinally sliced. Portions were routinely fixed in Zenker's acetic fluid and stained with hematoxylin and eosin as well as the periodic acid-Schiff technique. In addition, blocks of kidney from varying numbers of animals were fixed and processed according to the requirements of the following techniques as outlined in Lillie's "Histopathologic Technic and Practical Histochemistry,"[13] unless otherwise indicated.

Lipid:

1. Oil red O for neutral lipids (frozen sections; 10% formalin; 15 rats).

2. Schultz modification of Liebermann-Burchard technique for demonstration of cholesterol and/or esters (frozen sections; 10% formalin; 15 rats).

3. Windaus digitonin for free cholesterol (frozen sections; 10% formalin; 15 rats).

4. Acid hematein for phospholipids (calcium-formalin; 15 rats).

Enzymes:

1. Lipase with sorbitan monostearate (Tween 60) substrate (frozen sections; cold 10% formalin; 15 rats).

2. Glucose-6-phosphatase[14] (fresh frozen; sectioned in cryostat; 15 rats).

3. Succinic dehydrogenase[15] (fresh frozen; sectioned in cryostat; 60 rats).

4. G-nadi oxidase (fresh frozen; sectioned in cryostat; 60 rats).

5. Alkaline phosphatase (65% alcohol; 15 rats).

6. Acid phosphatase (acetone; 15 rats).

7. 5-Nucleotidase[16] (95% alcohol; 15 rats).

8. Nonspecific esterase[17] (frozen sections; cold 10% formalin; 15 rats).

Basement Membrane Stains:

1. Periodic acid-Schiff (routine).

2. Ritter-Oleson (combined periodic acid-Schiff and colloidal iron) (25 rats).

3. Periodic acid-methenamine silver[18] (25 rats).

*Furnished by Dr. Stanton Hardy, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

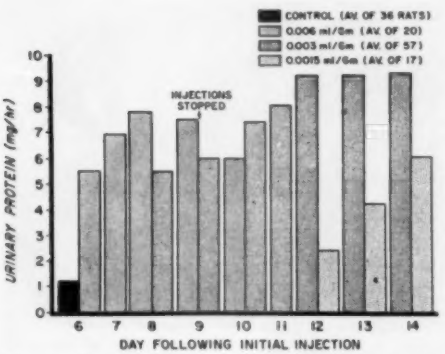
CONTROL (AV. OF 36 RATS)
0.006 ml/Gm (AV. OF 20)
0.003 ml/Gm (AV. OF 57)
0.0015 ml/Gm (AV. OF 17)
INJECTIONS STOPPED
URINARY PROTEIN (mg/hr)
DAY FOLLOWING INITIAL INJECTION

Fig. 1.—Degree and day of onset of proteinuria.

Other:

1. Phosphotungstic acid hematoxylin for mitochondrial elements (35 rats).

Oxygen Consumption of Kidney Slices.—The oxygen consumption of fresh kidney slices from kidneys of 21 rats receiving 0.003 ml. of aminonucleoside per gram and killed 14 days after the initial injection, 8 killed after six injections of a similar dose, and 13 controls were estimated by the standard manometric technique in a Warburg vessel, as described in detail in a previous report.[19]

Results

Clinical and Chemical Findings.—No clinical or biochemical alterations were encountered in rats receiving intravenous injections of aminonucleoside. Proteinuria (Fig. 1) appeared generally in 8 days (range, 7 to 12) after the initial injection of 0.003 ml. of aminonucleoside per gram. On the other hand, proteinuria appeared usually after 6 days (range, 5 to 9) in animals receiving 0.006 ml. per gram and at 12 days (range, 11 to 14) in animals receiving only 0.0015 ml. per gram.

Ascites frequently as great as 40 cc. and hydrothorax were evident in 52 of the 54 animals receiving nine injections of 0.003 ml. of aminonucleoside per gram and killed 14 days after the initial injection, in all animals receiving 0.006 ml. per gram and in only 4 of 18 rats receiving 0.0015 ml. per gram. Ascites when present appeared clinically, in most instances, two days after the onset of proteinuria.

The nonprotein nitrogen of the blood was elevated in all animals receiving aminonucleoside which developed proteinuria and in which this determination was performed, appearing most marked in those animals receiving the highest dosage employed. Animals receiving aminonucleoside also revealed an increase of cholesterol and neutral lipid, whereas total protein was decreased (Table 1).

Weight.—Animals receiving aminonucleoside revealed a gain in body weight similar to that of untreated controls despite the presence of ascites. Loss of weight was observed in rats receiving the 0.006 ml. per gram dose of the drug (Table 1).

Electrophoretic Findings.—A decrease in albumin and elevation of α-globulin was apparent in electrophoretic patterns obtained from the serum of rats receiving 0.003 ml. of aminonucleoside per gram. A marked increase in albumin was evident in the urine of these animals (Fig. 2).

Survival After Injection of Aminonucleoside.—Only 5 of 78 rats receiving 0.003 ml. of aminonucleoside per gram, or 6.4%, died within 14 days after the initial injection, whereas 13 of 16, or 81%, died between the 14th and 20th days after injection. Animals which survived failed to reveal clinical, biochemical, or morphologic alterations. All animals receiving 0.006 ml. per gram died within 11 days, whereas no

TABLE 1.—*Average Change in Body Weight and Biochemical Findings After Administration of Aminonucleoside*

Dose, Ml./Gm.	Day Killed	No.	Change in Wt., Gm.	N. P. N., Mg./100 Cc.	Cholesterol, Mg./100 Cc.	Total Lipid, Mg./100 Cc.	Total Protein, Gm./100 Cc.
0.003	14	35	+20.0	58.3	301	1400	2.5
0.003	6	16	+9.8 *	27.4	180	270	5.1
0.006	10	20	−19.0 †	94.0	280	670	4.3
0.0015	14	20	+23.0	38.0	210	470	4.8
Controls		36	+23.2	29.7	160	250	5.3

* Control weight (6 days) + 10 gm.
† Control weight (10 days) + 12 gm.

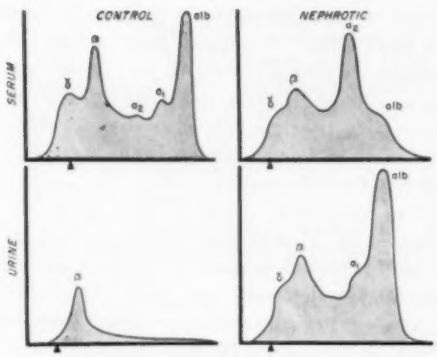

Fig. 2.—Electrophoretic patterns of pooled serum and urine from rats with aminonucleoside nephrosis and from untreated controls.

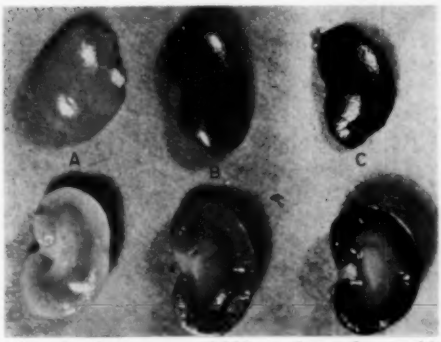

Fig. 3.—Appearance of kidneys from *A*, rat with aminonucleoside nephrosis; *B*, control killed 24 hours after injection of Evans blue dye. The cortex is only lightly stained by the dye, as compared to *C*, that of a rat with aminonucleoside nephrosis killed 24 hours after injection of Evans blue dye.

fatality was observed in animals receiving only 0.0015 ml. per gram. In these latter, proteinuria ceased about 30 days after its onset, and no biochemical or morphologic alterations were observed.

Protein Reabsorption.—Urine from animals receiving 0.003 ml. of aminonucleoside per gram and Evans blue dye on the 13th experimental day was stained with the dye, and the renal cortices of these animals were similarly colored. Frozen sections of such kidneys revealed numerous blue droplets in the cytoplasms of epithelial cells of the proximal convoluted tubules. The kidneys and urine of rats that received only five injections of this dose of aminonucleoside before the intravenous administration of Evans blue dye appeared similar to those of controls, being relatively unstained (Fig. 3).

Oxygen Consumption of Kidneys with Aminonucleoside Nephrosis.—A statistically significant depletion in the oxygen consumption of fresh kidney slices was apparent in rats with the nephrotic syndrome produced with aminonucleoside and has been described in detail elsewhere.[19] No alteration from normal was observed in animals killed

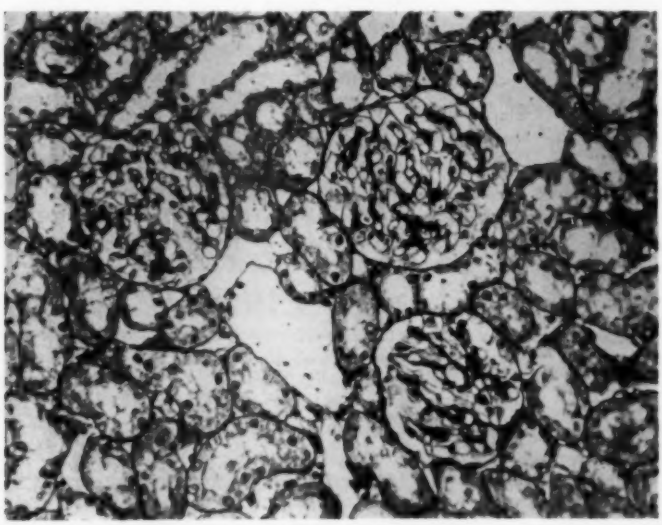

Fig. 4.—Glomeruli from nephrotic rat stained by the periodic acid-methenamine silver technique. There is no perceptible glomerular alteration. Reduced 20% from mag. × 300.

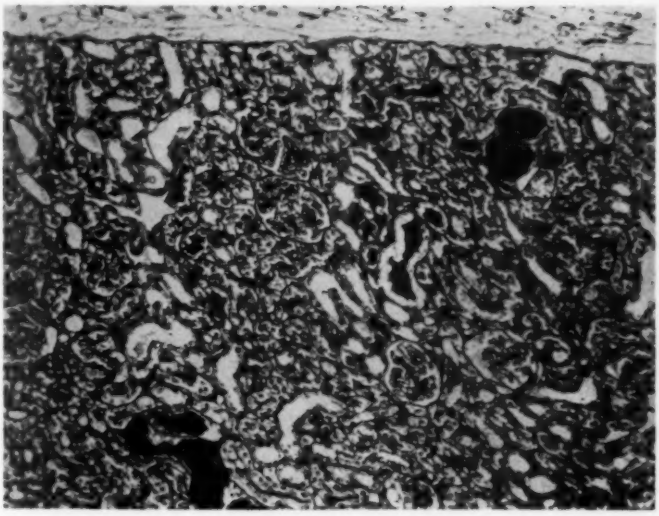

Fig. 5.—Section of kidney from rat with aminonucleoside nephrosis revealing moderate internal hydronephrosis and hyaline casts. Periodic acid-Schiff stain; reduced 20% from mag. × 150.

after only five injections. The addition of aminonucleoside in vitro failed to alter oxygen consumption of normal kidney slices.

Morphologic Observations.—Sections of kidneys from rats with the nephrotic syndrome resulting from aminonucleoside administration failed to reveal any alteration of the glomerular basement membranes, as evidenced in sections stained by the periodic acid-Schiff, periodic acid-methenamine silver, or Ritter-Oleson techniques (Fig. 4). On the other hand, the tubules frequently appeared dilated and their lumens contained hyaline casts which were strikingly demonstrated by the periodic acid-Schiff technique (Fig. 5). This method also revealed moderate numbers of "hyaline droplets" within the cytoplasms of epithelial cells of the proximal convoluted tubules. Mitochondrial elements demonstrated by the phosphotungstic acid hematoxylin method appeared significantly depleted in proximal convoluted tubules and ascending limbs of Henle. Droplets of neutral lipid were also encountered in the cytoplasms of epithelial cells of the proximal convoluted tubules and rarely within endothelial cells of glomerular

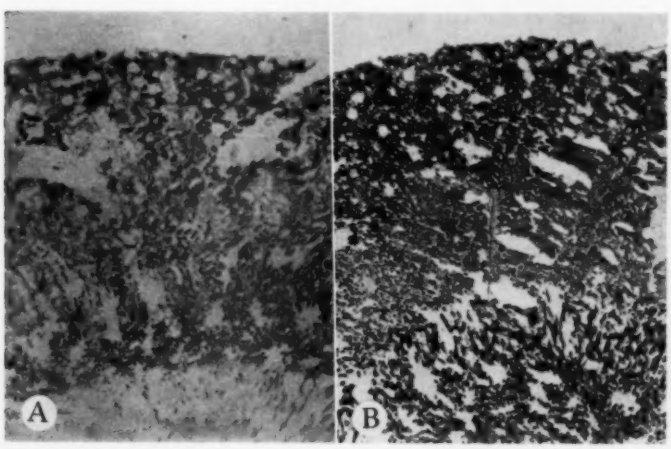

Fig. 6.—*A*, depletion of tubular succinic dehydrogenase in kidney from nephrotic rat, as compared to control, *B*. Reduced 15% from mag. × 35.

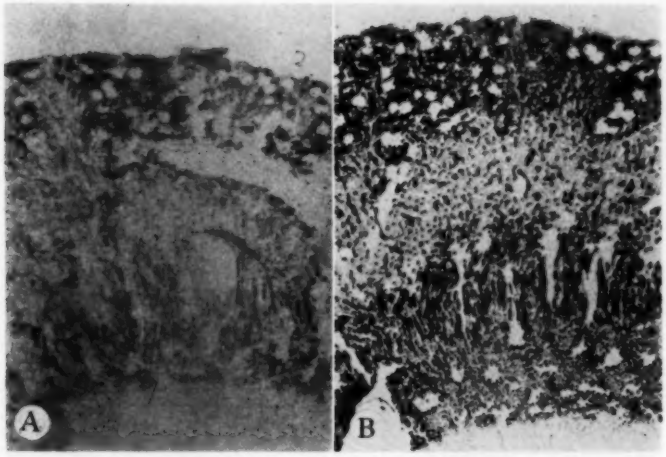

Fig. 7.—*A*, depletion of tubular cytochrome oxidase in kidney from nephrotic rat, as compared to control, *B*. Reduced 15% from mag. × 35.

tufts. Cholesterol and/or esters were not identified, and phospholipid was evident only in "hyaline droplets" and mitochondria. Animals killed after only five subcutaneous injections of 0.003 ml. of aminonucleoside per gram or one or two intravenous injections failed to reveal morphologic alteration. Similarly, the kidneys from animals exhibiting a remission from the nephrotic syndrome which were killed 45 days after the initial injection of the drug were histologically normal.

Tubular Enzymes.—Rats exhibiting the nephrotic syndrome after the injection of aminonucleoside revealed a significant depletion of histochemically demonstrable succinic dehydrogenase and cytochrome oxidase within the outer cortical and outer medullary zones of the kidney corresponding to the epithelial cells of the proximal convoluted tubules and ascending limbs of Henle (Figs. 6 and 7). There were no perceptible alterations in lipase; nonspecific esterase; glucose-6, acid, and alkaline phosphatase, or 5-nucleotidase in nephrotic rats. Animals which failed to develop the nephrotic syndrome did not reveal alteration in any of the enzymes studied.

The severity of depletion of tubular cytochrome oxidase and succinic dehydrogenase appeared directly related to the degree of proteinuria (Fig. 8). This was evident when estimated on the basis of degree of proteinuria on the day of killing, or average degree of protein excreted during the entire proteinuric period.

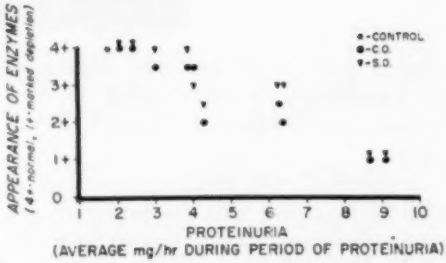

Fig. 8.—Correlation of degree of proteinuria and alteration of renal cytochrome oxidase and succinic dehydrogenase.

Comment

The results of this study indicate that the consistent development of the nephrotic syndrome in adult rats receiving aminonucleoside is dependent upon the dose utilized. An optimum dose for such an effect has been established as nine consecutive subcutaneous injections of 0.003 ml. of a 0.5% solution per gram. The severity of the disease induced in this manner is reflected by a survival rate of only 19% for longer than one week after the onset of nephrotic manifestations. Further, this state is of relatively short duration, with apparently

complete clinical, biochemical, and morphologic remission becoming evident within four weeks in animals which survive. Smaller doses of the drug produced ascites in only approximately 25% of the animals, and larger doses resulted in more rapid fatality.

Despite the absence of a demonstrable glomerular lesion, certain physiologic considerations [20] indicate that an alteration of the basement membranes of glomerular tufts, allowing for an abnormal degree of protein filtration, most likely exists in the nephrotic state produced by aminonucleoside. The increased tubular reabsorption of protein evidenced by the macroscopic and microscopic appearance of the kidneys after the injection of Evans blue dye precludes the consideration that the proteinuria observed in aminonucleoside nephrosis is the result of primary tubular dysfunction, since the results are unlike that after the injection of such tubular toxins as uranyl acetate [21] or mercury bichloride. Further, the distribution of dye in aminonucleoside nephrosis is similar to that noted in rats with the nephrotic state induced by the injection of anti-rat-kidney rabbit serum [21] in which definite alterations of the glomerular basement membranes may be easily recognized. In the light of the recent studies by Farquhar and associates,[22] it appears likely that such a lesion of glomerular basement membranes or podocytes might be appreciated in preparations examined by ultramicroscopic techniques. These investigators noted alteration of glomerular podocytes and basement membranes in sections of kidney from a patient with the nephrotic syndrome by electron microscopy, although glomerular lesions were not apparent with light microscopy.

The depletion of mitochondrial elements and respiratory enzymes within the epithelial cells of the proximal convoluted tubules and ascending limbs of Henle is similar to that observed in rats with the nephrotic syndrome induced by injection of highly potent and anti-rat-kidney rabbit serum.[9] It appears highly significant that the degree of depletion could be correlated with the degree of proteinuria experienced by these animals. The failure of aminonucleoside to alter the oxygen consumption of kidney slices from normal rats in vitro further indicates that the depletion of these enzymes is not the result of a direct tubular effect of this drug.[19] This information suggests that the reduction of these enzymes may represent either incomplete "exhaustion" due to increased tubular reabsorption of protein which occurs during the nephrotic state or represent a "toxic" effect of protein per se. The presence of a normal distribution and appearance of these enzymes and mitochondria in animals which have survived the

TABLE 2.—*Comparison of Nephrosis Due to Anti-Rat-Kidney Rabbit Serum (NTS) and Aminonucleoside (AN)*

	NTS *	AN
Nature of reaction	Antigen-antibody	Toxicity
Method of production	1 injection	Multiple injections
Onset of proteinuria	Immediate	6–14 days †
Degree of proteinuria	200 mg/18 hr.	100–200 mg./18 hr.
Body weight	Increased	No increase or loss †
Ascites	Massive	Massive †
Azotemia	Slight to moderate	Moderate to severe †
Hyperlipemia	Marked	Marked †
Hypoproteinuria	Moderately severe	Moderately severe †
Serum electrophoresis	Albumin dec.; α-globulin inc.	Albumin dec.; α-globulin inc.
Glomerular changes	Altered basement membrane	Apparently normal (light microscopy)
Tubular changes	Casts, hyaline droplets	Casts, hyaline droplets
Depletion of tubular enzymes	Cytochrome oxidase and succinic dehydrogenase	Cytochrome oxidase and succinic dehydrogenase
Depletion of mitochondria	Yes	Yes
Lipid in tubular epithelium	Cholesterol, neutral	Neutral only
Tubular reabsorption of protein (Evans blue dye)	Increased	Increased
Survival after onset of nephrosis	65%–80%	5%–100% †
Healed stage	Morphologic alteration	No morphologic residual

* Injection of highly potent serum.
† Depends on dose.

nephrotic state signifies the reversibility of these changes.

The lack of alteration in tubular lipase and nonspecific esterase in nephrosis induced by aminonucleoside is also similar to that observed after the injection of nephrotoxic serum [9] and supports recent observations [23,24] relating the hyperlipemia of the nephrotic state to abnormalities of renal excretory rather than enzymatic or hormonal function.

A comparison of the various clinical, biochemical, and morphologic features of the nephrotic states induced by anti-rat-kidney rabbit serum and aminonucleoside is tabulated in Table 2. The lack of apparent glomerular alteration in aminonucleoside nephrosis undoubtedly accounts for the failure to encounter "healed" lesions as in nephrotoxic serum nephrosis. The greater degree of proteinuria in the latter may also be related to the more apparent membranous alteration found in rats with nephrotoxic serum nephrosis. In the light of recent studies cited, it is most likely that the greater degree of hyperlipemia in rats with nephrotoxic serum nephrosis is the result of severer proteinuria experienced by these animals. The greater fatality rate and relative or absolute loss of body weight induced by aminonucleoside in the absence of relatively significant anatomical alterations in the kidney suggests that this agent may exert other toxic action or that the disease state produced by its administration may be accompanied by other unrecognized metabolic or enzymatic abnormalities, as suggested by Fiegelson and associates.[8] Despite the differences between the nephrotic states induced by aminonucleoside and by nephrotoxic serum, the similarities indicate the effectiveness of the two diverse agents in producing the nephrotic state in rats—a situation which is comparable to the varied etiologies which may be responsible for the nephrotic syndrome in man.

Summary

Appropriate doses of aminonucleoside (0.003 ml. of a 0.5% aqueous solution per gram subcutaneously for nine days) consistently produced nephrosis in mature rats, characterized by massive proteinuria, hypoproteinemia, hyperlipemia, and ascites. The severity of the disease was reflected by a fatality of 81% within one week after its onset. Complete clinical, biochemical, and morphological recovery was noted in four to five weeks in surviving animals. Smaller doses (0.0015 ml. per gram) of the drug produced the complete nephrotic syndrome less consistently, although survival and recovery was 100%. The largest dose of aminonucleoside tested (0.006 ml. per gram) also consistently produced nephrosis but was associated with death of all animals within one week after onset.

The renal lesion produced by aminonucleoside is characterized by tubular dilatation, hyaline casts, and hyaline droplets within tubular epithelium. Significant glomerular change was not appreciated in sections examined by light microscopy, although the degree of proteinuria, tubular lesions, and results of studies concerned with the tubular reabsorption of protein strongly suggest the presence of some alteration of the glomerular filtration apparatus.

Depletion of histochemically demonstrable succinic dehydrogenase and cytochrome oxidase and reduction of mitochondria was observed in cells of the proximal convoluted tubules and ascending limbs of Henle in rats with aminonucleoside nephrosis. The oxygen consumption of kidney slices from these rats was similarly depressed. The degree of histochemical alteration of these enzymes could be correlated with the severity of proteinuria. Possible mechanisms accounting for this enzymatic change are discussed.

The clinical, biochemical, and morphologic findings in the nephrotic states induced by the administration of aminonucleoside and highly potent anti-rat-kidney rabbit serum are compared.

Mr. John Colowit provided technical assistance.

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REFERENCES

1. Porter, J. N.; Hewitt, R. I.; Hesseltine, C. W.; Krupka, G.; Lowery, J. A.; Wallace, W. S.; Bohonos, N., and Williams, J. H.: Achromycin: A New Antibiotic Having Trypanocidal Properties, Antibiotics & Chemother. 2:409, 1952.

2. Taylor, D. J.; Bond, H. W., and Sherman, J. F.: Puromycin: I. Activity Against Experimental Amebiasis, in Antibiotics Annual 1954-1955, edited by H. Welch and F. Marti-Ibañez, New York, Medical Encyclopedia, Inc., 1955, pp. 745-750.

3. Hewitt, R. I.; Wallace, W. S.; Grumble, A. R.; Gill, E. R., and Williams, J. H.: Experimental Chemotherapy of Trypanosomiasis: III. Effect of Achromycin Against Trypansoma Equiperdum and Trypanosoma Cruzi, Am. J. Trop. Med. 2:254, 1953.

4. Tobie, E. J.: The Effect of Puromycin on 6 Species of Trypanosoma in Mice, Am. J. Trop. Med. 3:852, 1954.

5. Wright, J. C.; Dolgopol, V. B.; Logan, M.; Prigot, A., and Wright, L. T.: Clinical Evaluation of Puromycin in Human Neoplastic Disease, A. M. A. Arch. Int. Med. 96:61, 1955.

6. Troy, W.; Smith, S.; Personeus, G.; Moser, J.; Sparks, S.; Stevens, M.; Halliday, S.; McKenzie, D., and Oleson, J.: The Effects of Puromycin on Experimental Tumors, in Antibiotics Annual 1953-1954, edited by H. Welch and F. Marti-Ibañez, New York, Medical Encyclopedia, Inc., 1954, pp. 186-190.

7. Frenk, S.; Antonowicz, I.; Craig, J. M., and Metcoff, J.: Experimental Nephrotic Syndrome Induced in Rats by Aminonucleoside: Renal Electrolytes and Body Electrolyte Composition, Proc. Soc. Exper. Biol. & Med. 89:424, 1955.

8. Fiegelson, E. B.; Drake, J. W., and Recant, L.: Experimental Aminonucleoside Nephrosis in Rats, J. Lab. & Clin. Med. 50:437, 1957.

9. Fisher, E. R., and Gruhn, J. G.: Histochemical Observations Concerning Some Renal Enzymes in Nephrotoxic Nephrosis in the Rat, A. M. A. Arch. Path. 64:664, 1957.

10. Looney, J. M., and Walsh, A. I.: Determination of Spinal Fluid Protein with Photoelectric Colorimeter, J. Biol. Chem. 127:117, 1939.

11. de la Huerga, J.; Yesinick, C., and Popper, H.: Estimation of Total Serum Lipids by a Turbidimetric Method, Am. J. Clin. Path. 23:1163, 1953.

12. Creed, D. L.; Fisher, E. R., and Baird, W. F.: Paper Electrophoresis of Serum: Methods and Application, Pennsylvania M. J. 60:972, 1957.

13. Lillie, R. D.: Histolopathologic Technic and Practical Histochemistry, Ed. 2, New York, The Blakiston Company (division of McGraw-Hill Book Company, Inc.), 1954.

14. Chiquoine, A. D.: The Distribution of Glucose-6-Phosphatase in the Liver and Kidney of the Mouse, J. Histochem. 1:429, 1953.

15. Rosa, C. G., and Velardo, J. T.: Histochemical Demonstration of Succinic Dehydrogenase Activity in Tissue Sections by a Modified Technique, J. Histochem. 2:110, 1954.

16. McManus, J. F. A.; Lupton, C. H., Jr., and Harden, G.: Histochemical Studies of 5-Nucleotidase: Method and Specificity, Lab. Invest. 1:480, 1952.

17. Pearse, A. G. E.: Histochemistry: Theoretical and Applied, Boston, Little, Brown & Company, 1953.

18. Jones, D. B.: Nephrotic Glomerulonephritis, Am. J. Path. 33:313, 1957.

19. Fisher, E. R.; Tsuji, F. I., and Gruhn, J.: Effect of Nephrosis on Renal QO_2 and Histochemical Appearance of Cytochrome Oxidase and Succinic Dehydrogenase, Proc. Soc. Exper. Biol. & Med., to be published.

20. Sellers, A. L.; Griggs, N.; Marmorston, J., and Goodman, H. C.: Filtration and Reabsorption of Protein by the Kidney, J. Exper. Med. 100:1, 1954.

21. Goodman, H. C., and Baxter, J. H.: Tubular Resorption of Protein in Experimentally Produced Proteinuria in Rats, Proc. Soc. Exper. Biol. & Med. 93:136, 1956.

22. Farquhar, M. G.; Vernier, R. L., and Good, R. A.: Studies on Familial Nephrosis: II. Glomerular Changes Observed with the Electron Microscope, Am. J. Path. 33:791, 1957.

23. Rosenman, R. H.; Friedman, M., and Byers, S. O.: The Causal Role of Plasma Albumin Deficiency in Experimental Nephrotic Hyperlipemia and Hypercholesteremia, J. Clin. Invest. 35:522, 1956.

24. Di Luzio, N. R., and Houck, C. R.: The Role of the Kidney in the Etiology of Renal Hyperlipemia, J. Clin. Invest. 35:1381, 1956.

The Pathologic Physiology of Microscopic Pulmonary Vascular Shunts

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This report deals with a very unusual disorder of the pulmonary circulation characterized by the presence of striking proliferative and obliterating intimal alterations affecting small vessels of the lung. Such disorders of the small vessels of the lesser circulation are very rare and have been infrequently outlined in the American literature. The cases under discussion have one common denominator, namely, the finding of "shunts" between muscular pulmonary arteries and small vascular channels resembling veins. We feel, as will be subsequently outlined, that when such "shunts" undergo obliteration, the circulatory dynamics are sufficiently upset to cause cardiorespiratory failure and death.

Report of Cases

CASE 1.—A 13-month-old white girl was admitted to the hospital for the second time, having been diagnosed as having bronchitis associated with congenital heart disease. The child was born after an uncomplicated pregnancy, and the birth weight was 7 lb. 4 oz. When the baby was 7 days of age the mother noted rapid respirations and some cyanosis. At age 3 months there occurred a severe bout of cyanosis, unconsciousness, and very slow respirations. A diagnosis of possible congenital heart disease was made.

The first hospital admission was at age 7 months, for cardiac evaluation. The heart was enlarged by percussion to the left midclavicular line. There was a soft blowing systolic murmur heard best in the left third or fourth interspace, with transmission to the apex. Chest x-ray showed bilateral ventricular enlargement. ECG showed right ventricular hypertrophy and possible left ventricular hypertrophy.

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On the final admission the infant had had a "cold" for three weeks. During these three weeks there had occurred three episodes of cyanosis and irritability and she was eating poorly. She was afebrile. Physical examination was as on the first admission. Seventeen hours after admission respirations ceased.

Gross Autopsy Findings.—Slight lip cyanosis was present. The heart weighed 90 gm. There were a defect of the membranous interventricular septum 0.4 cm. in diameter, a bicuspid aortic valve, and marked right ventricular hypertrophy, with dilatation of the pulmonary artery and its main branches. The foramen ovale admitted a slender probe, and the ductus arteriosus was virtually obliterated. The lungs were crepitant, and no evidence of congestion, edema, hemorrhage, or pneumonia was found. The remaining organs, including the brain, were unremarkable.

CASE 2.—A 22-month-old white girl was admitted to the hospital with a history of repeated "fainting spells" for approximately four weeks. During the last two episodes she had apparently been unconscious for several minutes.

The infant had been fed solid foods until aged 5 months but had been fed virtually milk alone since then. There were no previous illnesses. Physical examination revealed pallor and a degree of malnutrition.

Hemoglobin was 5.8 gm. per 100 cc.; erythrocytes were 3,200,000 per cubic millimeter. There was 1+ albuminuria.

On the second hospital day a blood transfusion of 220 cc. was begun slowly. Two hours later a fainting episode occurred. Respirations became gasping, and death ensued rapidly. Repeat compatibility tests revealed no incompatibility.

Gross Autopsy Findings.—The right subclavian artery originated from the aortic arch on the left below the origin of the left subclavian artery and crossed to the right behind the esophagus.

The heart weighed 80 gm. The ductus arteriosus was closed; no septal defects were present. The right ventricle was markedly hypertrophied, and the endocardium, normal. There was coagulated milk in both main bronchi and in the trachea.

Approximately 20 cc. of ascitic fluid was present. The remaining organs, including the brain, were unremarkable.

CASE 3.—An 11-month-old white girl was admitted to the hospital for elective repair of a congenital harelip.

At birth the infant manifested numerous congenital abnormalities, among which were harelip, left complete cleft palate, and meningomyelocele with spina bifida.

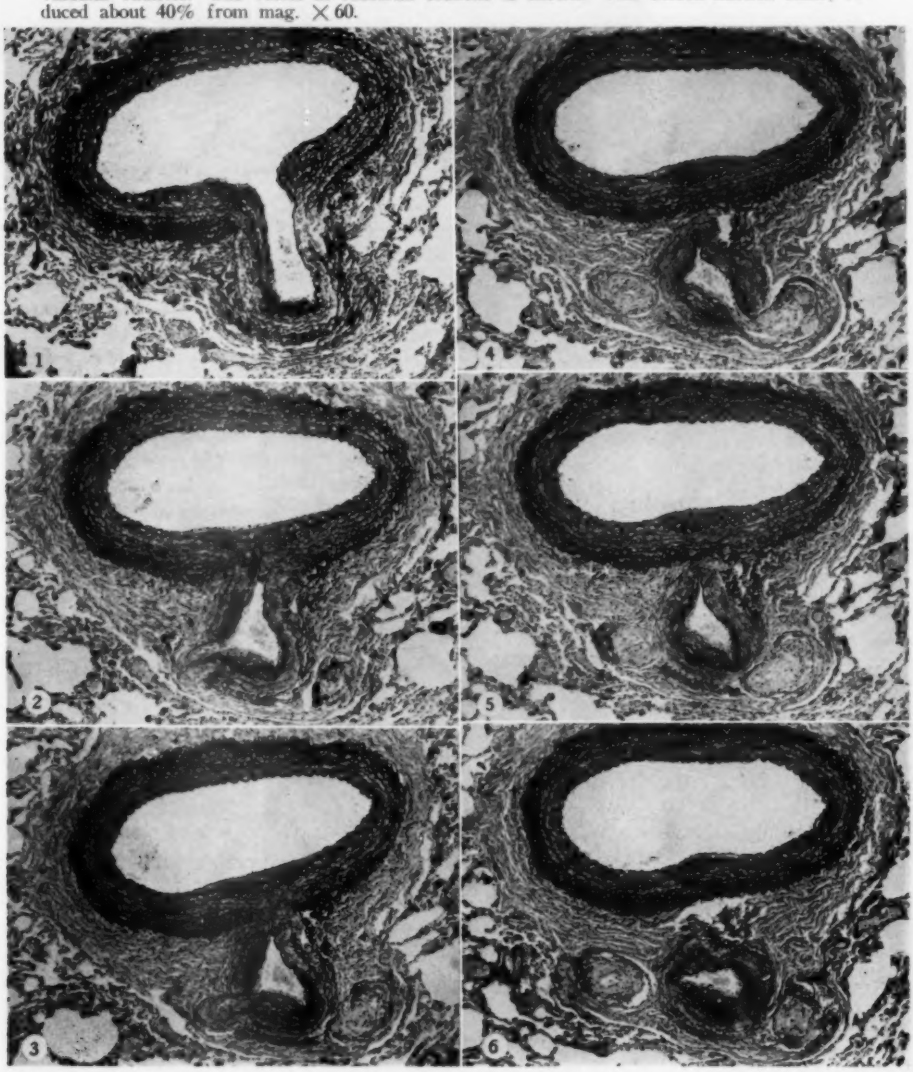
The infant showed gradual but progressive weight gain and development. A previous admission at age 4 months for repair of the harelip resulted in deferment of surgery because of difficulty in intubation during anesthesia.

Since the first admission the infant had had frequent colds, chickenpox, and, more recently, seborrheic dermatitis with a yeast infection of the skin.

Physical examination showed bilateral colobomata, with minimal visual acuity, unilateral cleft lip, and Grade 4 cleft palate.

The pulse rate was 100. Systolic murmurs were heard over the mitral, aortic, and pulmonic areas.

Figs. 1-6.—Medium-sized artery, illustrating obliteration of the lumina of secondary vascular radicles. One vessel in subserial sections is shown. Van Gieson-elastica stain; reduced about 40% from mag. × 60.



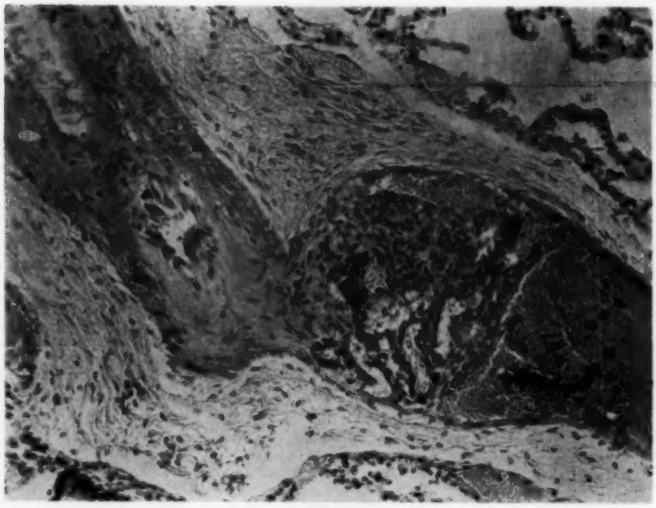


Fig. 7.—Cellular intimal proliferation between artery and dilated thin-walled vascular channel. Hematoxylin and eosin.

The apex beat was outside the limits of normal. The lungs were normal.

There was a lumbar meningomyelocele, with spasticity of the lower limbs with paresis.

Urinalysis showed a trace of albumin. The hemoglobin was 12.6 gm. per 100 cc.; the white blood cell count, 22,700, with 58% neutrophils, and there were 4 nucleated red blood cells per 100 white blood cells.

The harelip was repaired uneventfully, but on the first postoperative day the temperature rose to 103 F (rectal), and the infant died on the following day.

Gross Autopsy Findings.—The above-listed congenital anomalies were noted, and, in addition, there was bilateral dislocation of the hips. The meningomyelocele contained nerve filaments and 100 cc. of clear colorless fluid.

The heart weighed 74 gm. The left and right ventricles measured 0.6 and 0.8 cm. in thickness, respectively, indicating a marked right ventricular hypertrophy. The foramen ovale admitted a slender probe.

There was a 1.5 cm. interventricular septal defect in the area of the septum membranaceum. The ductus arteriosus was widely patent. The valves were normal.

The lungs were subcrepitant. Some curdled milk was present in the larynx and trachea.

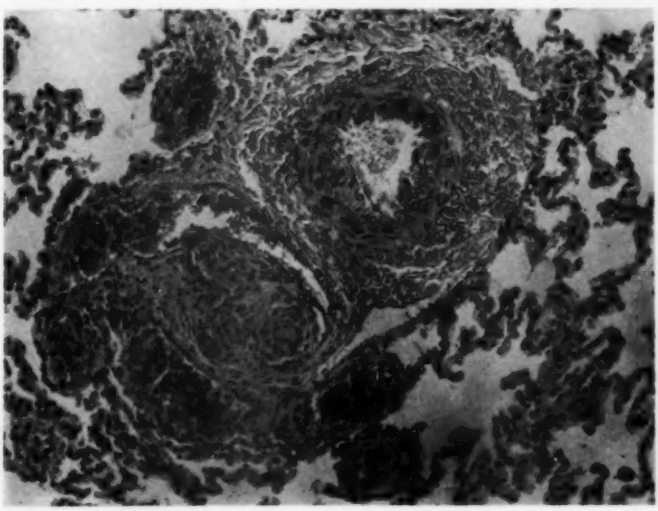


Fig. 8.—Intimal plaque in artery adjacent to obliterating channel. Note dilated channels rimming the area. Hematoxylin and eosin.

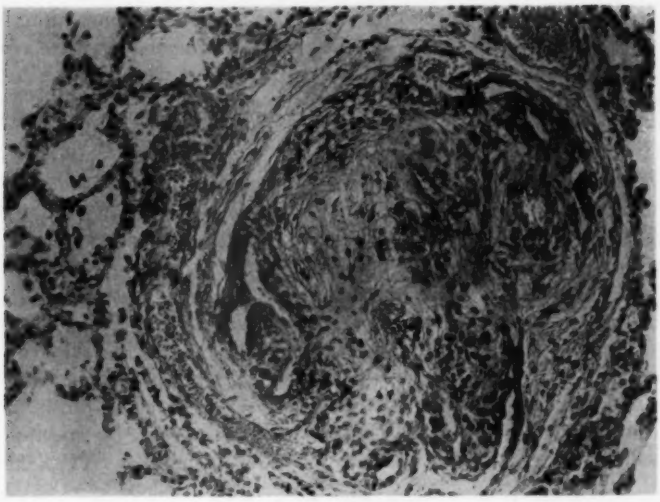


Fig. 9.—A complex fibrocellular lesion communicating with an artery. Hematoxylin and eosin.

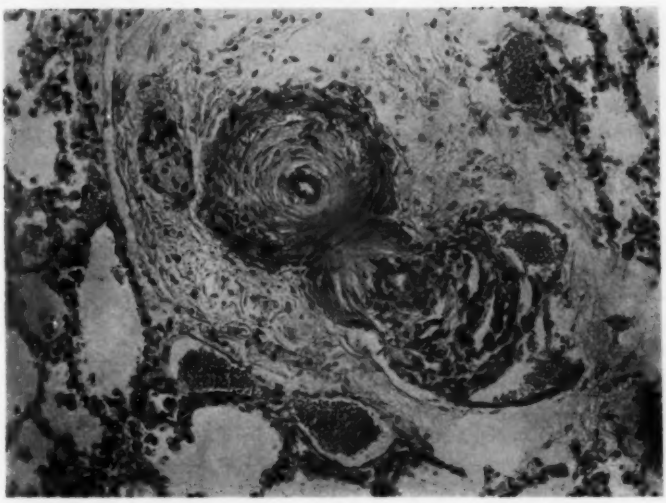


Fig. 10.—Arteriosclerotic vessel with branch showing endothelial proliferation and distal thin-walled channels. Hematoxylin and eosin.

The remaining organs were unremarkable, with the exception of the brain, which displayed an internal hydrocephalus, and the ileum showed a diverticulum 1 cm. in diameter.

Histologic Examination

Examination of the lungs of all three cases revealed similar findings and forms the basis of this report. Similar vascular changes were not observed in the other organs. The lesions involved small muscular arteries. The larger branches of the pulmonary vasculature showed no significant alterations. The arteries of approximately 1 to 2 mm. in diameter gave off branches which in turn led into smaller ramifications which showed extreme endothelial proliferation and obliteration. This obliterative process is illustrated in Figures 1 to 6 (Case 1). Distal to this occlusive process the elastica disappeared entirely and the vessel media thinned, only to give rise to a second occlusive proliferation of endothelial type cells which some have termed "glomoid" (Fig. 7 [Case 3]). Yet more distally, the

vessel entered into a series of dilated thin-walled channels, giving the area an angiomatous appearance, (Fig. 8 [Case 2], Fig. 9 [Case 3], Fig. 10 [Case 3]). Although these channels in limited serial sections could not be traced to true veins, they were in very close proximity to them. The arteries were always peribronchial in location, and the pattern was very constant. The original branching artery appeared to have given off several occluded branches, all displaying the above histologic alterations. There was no inflammatory component, and although agonal thrombus material may have been present in the lumen, stages of organization were not seen. Other small vessels usually showed medial hypertrophy and intimal fibrosis. Within the areas of endothelial proliferation no hematogenous blood pigment was found.

Comment

Aside from the distinctive morphology and obscure morphogenesis the lesion is of interest from two other standpoints—the relationship, if any, to experimentally demonstrated pulmonary arteriovenous shunts and the relationship, cause or effect, to primary and secondary pulmonary hypertension.

Many publications as far back as at least 1924 have illustrated this lesion, usually in single case reports.[1] Although a few cases are associated with the syndrome of primary pulmonary hypertension,[2-6,29] the majority have been accompanied by one of three cardiovascular defects, i. e., patent ductus arteriosus, interventricular septal defect, and interatrial septal defect, and thus may be termed cases of secondary pulmonary hypertension.[2,7-13] Case 3 in our series obviously falls into the secondary group, and Case 2, into the primary group. Case 1 is equivocal, since such a small interventricular septal defect (4 mm. in diameter) is usually considered of minor clinical consequence.

Although pulmonary arteriosclerosis and similar obstructive pulmonary lesions have been produced experimentally,[14,15] no lesions resembling the obliterative phenomena herein reported have been illustrated. Some authors have produced experimental pulmonary arteriosclerosis through the intravenous injection of blood clots.[14,15] The possibility of such an embolic pathogenesis for the lesion under discussion has not been considered seriously by the majority of those studying it, and in this we concur. The uniformity of appearance, including vessels involved and absence of stages of organization, militate against this concept, and sources of emboli were not found.

Additional evidence challenging this concept is contained in a report dealing with the production of severe arteriosclerosis of small pulmonary vessels merely by injection of air into the veins of animals. Thus the theory of organization of blood clots need not be invoked, and the elements of spasm and distention enter the picture.[16] In view of the complete absence of an inflammatory reaction in either the involved vessels or their adventitia, an infectious etiology would seem very unlikely.

Most reports either have been obscure as to pathogenesis or have favored a mechanical method of production. Concerning the latter, Edwards proposes that a loss of reserve in the pulmonary system, a system already characterized by medial hypertrophy, results in a right-to-left shunt secondary to which (in cases of congenital heart disease) fibrous intimal proliferation occurs as a second attempt to balance the circulation. The "glomoid" proliferation he sees as a possible reaction of endothelium to an irritant within the wall, i. e., hyalinized or fibrinoid material. His experience indicates that a time factor is necessary for their production and that they are rare under the age of 2, although all three of our cases are under this age. Regarding cases of primary pulmonary hypertension, the retention of the thick medial muscle of fetal arterioles may be the basic factor in initiating right ventricular hypertrophy, as described by Edwards, in 1950.[18]

Major interest of other authors [2,3,8,22] has centered about the possibility that these

lesions represent arteriovenous shunts undergoing obliteration. Spencer [3] has reported tracing the dilated thin-walled channels to pulmonary veins. Brewer,[9] although tracing them in one of many serial sections to a vein, does not believe them to be arteriovenous shunts. Edwards [10] has not been able to trace these obliterated vessels to veins and likewise does not believe them to represent such shunts.

The existence of pulmonary arteriovenous shunts in normal lungs has been neglected until reports by several authors using injection techniques clearly showed them to be present in animal and human lungs. Using a method of injecting glass beads into pulmonary arteries of human lungs, Tobin and Zariquiey [23,24] confirmed animal experiments of Prinzmetal [25] and others in which spheres up to 500μ in diameter were recovered from the pulmonary veins. Plastic injections indicated no shunts at the hilum but their presence at apices of lobular subdivisions and in the pleura.

Similar experiments by Niden and Aviado [26] on dogs confirmed shunts up to 420μ. In their experiments concerned with the effects of pulmonary embolism on the pulmonary circulation they noted that anoxemia was a conspicuous feature. They postulated that this anoxemia was caused by opening of preexisting arteriovenous communications and that the early relief of anoxemia noted in the experiments was brought about by their closure.

Some doubt has been cast upon the glass-sphere experiments by others.[27,28] Thus, further experimental work is necessary to establish the presence or absence of these shunts before the lesions under discussion can be fully understood in such a light.

Regarding the enigma of primary pulmonary hypertension, acceptable reports on infants illustrating the lesions herein described number very few in a fairly extensive survey of the literature.[5,6] The vast majority of reports of primary pulmonary hypertension illustrate only medial hypertrophy with or without intimal sclerosis.[19-21]

We have attempted in this paper to correlate our morphologic observations involving the pulmonary circulation with clinical findings and experimental physiologic studies. The pathology of the lesion under discussion involves medium-sized muscular branches of the pulmonary artery. It consists of the presence of "shunts" between these vessels and smaller nonmuscular vascular channels, which in our studies cannot be shown to communicate with veins on the basis of almost 100 serial sections. Two conclusions could be drawn in this respect. First, the "shunts" in reality are blindly ending pseudoangiomatous collaterals; second, beyond the point of obliteration the "shunts" collapse and become venules. We favor the latter interpretation in view of the experimental evidence involving perfusion studies of the lesser circulation outlined above.

The etiology of this condition is obscure. In cases under discussion a congenital factor seems difficult to eliminate, since all three cases had varying types of associated congenital malformations. An infectious or postinfectious etiology seems highly unlikely in view of the absence of any active inflammatory reaction and, secondly, the absence of fibrosis, hyalinization, or other stigmata of the inflammatory process. It would appear more likely that this entity represents a disturbance in the physiologic pathology of pulmonary "arteriovenous shunts" with associated vascular reaction, possibly of the type described by Edwards, with obliterative changes and eventual right-heart hypertrophy and failure. The occurrence of the condition in three children under 2 years of age is of interest in light of other observations in the literature, most of which involve older children and young adults.

Summary

Three cases are presented of a distinctive endoarterial obliterative vascular lesion in the lungs of infants showing at necropsy pronounced right-heart hypertrophy with and without congenital heart disease.

The obscure nature of these lesions and their relationship to possible arteriovenous anastomosis and to the syndrome of primary pulmonary hypertension are discussed.

The medical illustration section of the First United States Army Medical Laboratory, 90 Church St., New York 7, provided the photomicrographs of Cases 2 and 3.

Huntington Hospital (Dr. McNicol).

REFERENCES

1. zur Linden, W.: Isolierte Pulmonalsklerose im jüngsten Kindesalter, Arch. path. Anat. 252: 229, 1924.

2. Kucsko, L.: Über arteriovenöse Verbindungen in der menschlichen Lunge und ihre funktionelle Bedeutung, Frankfurt. Ztschr. Path. 64:54, 1953.

3. Spencer, H.: Primary Pulmonary Hypertension and Related Vascular Changes in the Lungs, J. Path. & Bact. 62:75, 1950.

4. Uschold, G.: Über einen Fall von Endarteritis pulmonalis unter dem Bild eines offenen Ductus Botalli bei einem 7½ jährigen Kind, Arch. klin. Chir. 271:17, 1952.

5. Chambers, V., and Wellington, J. S.: Pulmonary Endarteritis with Cor Pulmonale in Infancy, J. Pediat. 49:437, 1956.

6. Cross, K. R., and Kobayashi, C. K.: Primary Pulmonary Vascular Sclerosis: Report of Case, Am. J. Clin. Path. 17:155, 1947.

7. Wätjen: Zur Kenntnis der Pulmonalsklerose im Säuglingsalter, Verhandl. deutsch. Gesellsch. Path. 21:259, 1926.

8. Gordon, A. J.; Donoso, E.; Kuhn, L. A.; Ravitch, M. M., and Himmelstein, A.: Patent Ductus Arteriosus with Reversal of Flow, New England J. Med. 251:923, 1954.

9. Brewer, D. B.: Fibrous Occlusion and Anastomosis of the Pulmonary Vessels in a Case of Pulmonary Hypertension Associated with Patent Ductus Ateriosus, J. Path. & Bact. 70:299, 1955.

10. Edwards, J., in Henry Ford Hospital International Symposium on Cardiovascular Surgery: Studies in Physiology, Diagnosis and Techniques, edited by C. R. Lam, Philadelphia, W. B. Saunders Company, 1955.

11. Campbell, M., and Hudson, R.: Patent Ductus Arteriosus with Reversed Shunt Due to Pulmonary Hypertension, Guys Hosp. Rep. 100: 26, 1951.

12. Smith, G.: Patent Ductus Arteriosus with Pulmonary Hypertension and Reversed Shunt, Brit. Heart J. 16:233, 1954.

13. Rutishauser, E., and Blanc, W.: Anastomoses artério-veineuses glomiques du poumon avec syndrom d'insuffisance droite et cyanose, Schweiz. Ztschr. allg. Path. 13:61, 1950.

14. Harrison, C. V.: Experimental Pulmonary Arteriosclerosis, J. Path. & Bact. 60:289, 1948.

15. Muirhead, E. E., and Montgomery, P. O.: Thromboembolic Pulmonary Arteritis and Vascular Sclerosis: Its Experimental Production in Rabbits by Means of Intravenously Injected Human Amniotic Fluid and Autogenous Blood Clots, A. M. A. Arch. Path. 52:505, 1951.

16. Barnard, P. J.: Pulmonary Arteriosclerosis Due to Oxygen, Nitrogen, and Argon Embolism: Experimental Study, A. M. A. Arch. Path. 63:322, 1957.

17. Edwards, J. E.: The Lewis A. Conner Memorial Lecture, Functional Pathology of the Pulmonary Vascular Tree in Congenital Cardiac Disease, Circulation 15:164, 1957.

18. Edwards, J. E.: Structural Changes of the Pulmonary Vascular Bed and Their Functional Significance in Congenital Cardiac Disease: Twenty-Sixth Ludvig Hektoen Lecture, Proc. Inst. Med. Chicago 18:134, 1950.

19. Berthrong, M., and Cochran, T. H.: Pathological Findings in 9 Children with "Primary" Pulmonary Hypertension, Bull. Johns Hopkins Hosp. 97:69, 1955.

20. Goodale, F., Jr., and Thomas, W. A.: Primary Pulmonary Arterial Disease: Observations with Special Reference to Medial Thickening of Small Arteries and Arterioles, A. M. A. Arch. Path. 58:568, 1954.

21. Carpenter, H. M., and Prichard, R. W.: Primary Pulmonary Vascular Disease: Report of 5 Cases, Am. J. Clin. Path. 26:899, 1956.

22. Chiari, H.: Cor Pulmonale, Bull. schweiz. Akad. med. Wissensch. 6:432, 1950.

23. Tobin, C. E., and Zariquiey, M. O.: Arteriovenous Shunts in the Human Lung, Proc. Soc. Exper. Biol. & Med. 75:827, 1950.

24. Tobin, C. E., and Zariquiey, M. O.: Some Observations on the Blood Supply of the Human Lung, M. Radiog. & Photog. 29:9, 1953.

25. Prinzmetal, M.; Ornitz, E. M., Jr.; Simkin, B., and Bergman, H. C.: Arterio-Venous Anastomoses in Liver, Spleen, and Lungs, Am. J. Physiol. 152:48, 1948.

26. Niden, A. H., and Aviado, D. M., Jr.: Effects of Pulmonary Embolism on the Pulmonary Circulation with Special Reference to Arteriovenous Shunts in the Lung, Circulation Res. 4: 67, 1956.

27. Bostroem, B., and Piiper, J.: Über arteriovenöse Anastomosen und Kurzschlussdurchblutung in der Lunge, Arch. ges. Physiol. 261:165, 1955.

28. Gordon, D. B.; Flasker, J., and Drury, D. R.: Size of the Largest Arterio-Venous Vessels in Various Organs, Am. J. Physiol. 173:275, 1953.

29. Kuida, H.; Dammin, G. J.; Haynes, F. W.; Rapaport, E., and Dexter, L.: Primary Pulmonary Hypertension, Am. J. Med. 23:166, 1957.

Influence of Abdominal Radiation on Response of Rabbits to Intravenous Endotoxin

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Several experimental methods have been employed to produce fibrinoid lesions in rabbits. These include the intravenous administration of Gram-negative endotoxin,[1,2] alone or in association with the administration of other substances, such as cortisone,[3] colloidal iron or carbon,[4] trypan blue,[5] streptococci,[6] or certain high-molecular-weight acidic polymers, such as sodium polyanetholsulfonate (Liquoid).[7,8]

One of the characteristic changes produced by these procedures is bilateral renal cortical necrosis, and this lesion has been termed the "identifying" gross lesion of the generalized Shwartzman phenomenon.[9] The development of this lesion appears to be due to obstruction of the renal glomerular capillaries by an acidophilic homogeneous material with the tinctorial properties of fibrinoid. Hyaline material with similar morphologic and tinctorial features occurs also in the splenic sinusoids, in the hepatic veins, in the pulmonary arteries, and within the walls of the coronary arteries and substance of the cardiac valves.[2,8,10-13]

The similarity of these experimental fibrinoid lesions to the lesions found in human systemic fibrinoid diseases has been emphasized in other papers.[14-16] The lesions of radiation nephritis, described by Zuelzer,[17] Smith,[18] and Dean, and the morphologic changes observed in three recent cases of radiation nephritis examined in the University of Minnesota Department of Pathology suggested that the administration of endotoxin to irradiated rabbits might produce similar renal lesions. The present paper describes the lesions produced in rabbits by this method.

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From the Department of Pathology, University of Minnesota Medical School. Medical Fellow, American Cancer Society (Dr. Jarvis). Part of this work was done during the tenure of a Medical Student Fellowship of the National Foundation for Infantile Paralysis (Mr. Schrader). Senior Research Fellow, U. S. P. H. S. (Dr. Brunson).

Materials and Methods

Two hundred hybrid albino rabbits of both sexes, weighing 1 to 1.5 kg., were used in the experiments. They were fed Purina Rabbit Pellets and had free access to water.

Radiation was provided by a General Electric Maximar operated at 220 kv. and 15 ma., with a cone having an area of 100 sq. cm. at a focus-skin distance of 50 cm. A filter of one-fourth copper was employed, giving a half-value layer of 0.95 mm. of copper. The machine was calibrated by Victoreen Meter No. 378 and produced 67 r in air per minute. The depth dose to the kidneys at a depth of 4 cm., calculated on a basis of 100 sq. cm., was 95 tissue roentgens for each 100 r in air,[20] or 63.7 tissue roentgens per minute.

The rabbits were prepared by radiation to the abdomen, with the edge of the cone at the level of the costal margin. The cone covered approximately 80% of the abdomen. The animals were tied to a board, with their abdomens toward and perpendicular to the central beam of the x-ray tube during radiation. The field of radiation included the liver, the spleen, the kidneys, the gastrointestinal tract, the adrenals, and the lateral portions of the bases of both lungs. A single dose of x-ray was used on each animal. Direct radiation to the heart and as much of the lungs as was possible was avoided.

In conjunction with irradiation, at varying intervals of time, each of the animals was given a single intravenous injection of Gram-negative endotoxin. Appropriate controls were used in each instance. The number of animals used in the experiments and the various procedures carried out are summarized in the accompanying tables.

TABLE 1.—*Incidence of Fibrinoid Lesions in Rabbits Given Intravenous Endotoxin After 300 R in Air to the Abdomen*

Hr. Between Procedures	Animals, No.	Dying Within 12 Hr., No.	Microscopic Lesions				
			Kidney	Spleen	Liver	Heart	Lung
12	8	0	2	4	0	0	3
18	8	0	2	5	3	1	2
24	8	0	0	3	2	1	3
48	8	0	0	2	2	2	1
Controls (300 r in air without toxin)	8	0	0	1	0	0	2

Meningococcal endotoxin was used throughout the experiments. The details of its preparation have been reported previously.[9] It was diluted 1:80 with sterile, pyrogen-free, isotonic saline solution and injected in a volume of 2 cc. into the marginal ear vein of the rabbits. The animals died or were killed 24 hours after the last procedure performed. Postmortem examinations were performed, and the tissues were fixed in 10% neutral formalin. Sections were taken from the kidneys, spleen, liver, pancreas, adrenal glands, skeletal muscle, heart, lungs, and intestines. These were stained routinely with hematoxylin and eosin, but many additional sections were stained by the periodic acid-Schiff method, Mallory's phosphotungstic acid hematoxylin, and toluidine blue.

Results

The results are summarized in Tables 1-4. From these Tables it may be seen that with dosages of 300 or 600 in air followed by endotoxin there were no acute deaths. However, when the animals were given endotoxin in conjunction with 1200 r in air, 8 of 32 animals died within 12 hours after administration of the toxin, and of those given 2400 r in air, 5 of 32 died within 12 hours after endotoxin was given. It is of interest that in each of these groups the greatest incidence of deaths occurred when

TABLE 2.—*Incidence of Fibrinoid Lesions in Rabbits Given Intravenous Endotoxin After 600 R in Air to the Abdomen*

Hr. Between Procedures	Animals, No.	Dying Within 12 Hr., No.	Microscopic Lesions				
			Kidney	Spleen	Liver	Heart	Lung
12	8	0	2	5	1	0	2
18	8	0	1	1	0	1	4
24	12	0	8	9	6	2	9
48	8	0	0	3	2	1	3
Controls (600 r in air without endotoxin)	8	0	0	1	0	0	1

TABLE 3.—*Incidence of Fibrinoid Lesions in Rabbits Given Intravenous Endotoxin After 1200 R in Air to the Abdomen*

Hr. Between Procedures	Animals, No.	Dying Within 12 Hr., No.	Microscopic Lesions				
			Kidney	Spleen	Liver	Heart	Lung
12	8	5	3	8	2	1	3
18	8	1	4	6	3	2	2
24	8	0	6	8	4	3	6
48	8	2	4	6	4	1	8
Controls (1200 r in air without endotoxin)	8	0	0	1	0	0	0

TABLE 4.—*Incidence of Fibrinoid Lesions in Rabbits Given Intravenous Endotoxin After 2400 R in Air to the Abdomen*

Hr. Between Procedures	Animals, No.	Dying Within 12 Hr., No.	Microscopic Lesions				
			Kidney	Spleen	Liver	Heart	Lung
12	8	3	4	7	3	1	2
18	8	0	7	8	4	4	5
24	8	1	2	7	2	1	3
48	8	1	2	3	2	4	2
Controls (2400 r in air without toxin)	8	2	0	3	1	0	0

the interval between radiation and administration of endotoxin was 12 hours. It is also of interest (Table 4) that two animals of a control group of eight given 2400 r in air died within 12 hours after irradiation.

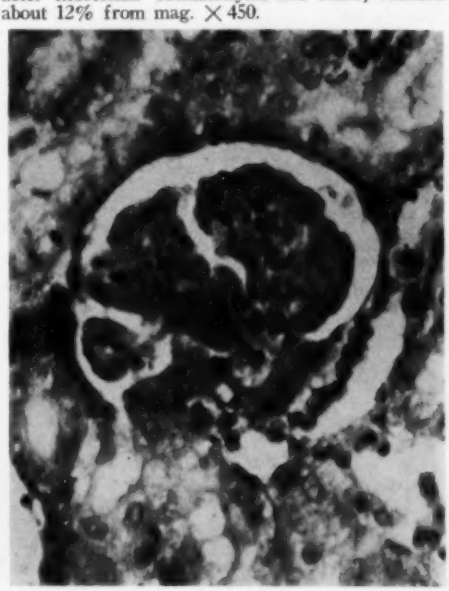
TABLE 5.—*Comparison of Results Obtained by Varying the Interval Between Injection of Endotoxin and Different Dosages of Irradiation*

Initial Procedure, R in Air	Interval, Hr.	2d Procedure	Animals, No.	Gross Renal Cortical Necrosis, No.	Microscopic Fibrinoid Lesions				
					Kidney	Spleen	Liver	Heart	Lung
300	18	Toxin	8	1	2	5	3	1	2
600	24	Toxin	12	2	8	9	6	2	9
1,200	24	Toxin	8	4	6	8	4	3	6
2,400	18	Toxin	8	0	7	8	4	4	5
Toxin	24	1,200 r in air	8	0	0	3	0	0	2
1,200	None	No toxin	8	0	0	1	0	0	0

The morphologic changes observed in the animals were similar in all groups but varied extensively in incidence, as shown in Tables 1-4. At each dose range of x-ray, the interval after which endotoxin was given appeared to alter the number of animals which developed lesions. In those given 300 r in air, for example, a greater number of animals developed lesions when the interval between x-ray and endotoxin was 18 hours. In the groups given 600 or 1200 r in air, the optimal interval was 24 hours, while in the group given 2400 r in air it was 18 hours. For comparison purposes, these optimal groups are summarized in Table 5.

The gross renal lesion consisted of bilateral cortical necrosis, strikingly similar to that observed in the generalized Shwartzman phenomenon. Microscopically, the glomerular capillaries were occluded by masses of homogeneous acidophilic hyaline material, and there was extensive hemorrhage and tubular necrosis (Fig. 1). The hyaline material stained magenta by the periodic acid-Schiff method, purple to orange-purple with phosphotungstic acid hematoxylin, and blue when stained with toluidine blue. Its morphologic and tinctorial

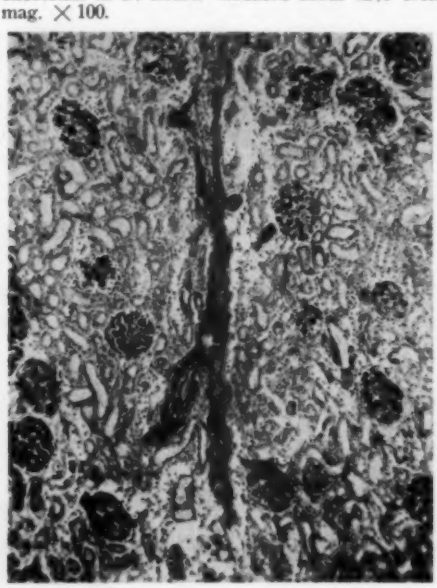
Fig. 1.—Section of a kidney, showing a glomerulus with fibrinoid material which occludes the capillaries. From a rabbit given 1200 r in air followed by endotoxin in 48 hours. Killed 24 hours after endotoxin. Hematoxylin and eosin; reduced about 12% from mag. × 450.



features, therefore, were similar to those of the hyaline fibrinoid material which occurs in the generalized Shwartzman phenomenon.[2,7,9]

In many animals no gross renal changes were observed, but microscopic examination showed that varying numbers of glomeruli contained fibrinoid material. In some glomeruli the material was closely applied to the capillary basement membranes, producing a thickening which resembled the "wire loops" of disseminated lupus erythematosus. In other glomeruli there were focal intraluminal accumulations of fibrinoid. Many of the glomeruli in these animals appeared enlarged and hypercellular, with an increased number of endothelial cells. In addition, it was observed that fibrinoid material was present in the lumens and walls of many of the interlobular arteries and afferent arterioles. These vessels were occasionally completely occluded by intraluminal masses of this material (Fig. 2).

Fig. 2.—Section from a kidney stained by the periodic acid-Schiff method, showing an interlobular artery with Schiff-positive fibrinoid material in the walls and lumen. Deposits of fibrinoid material are also evident in the glomerulus. From a rabbit given 1200 r in air followed by endotoxin in 24 hours. Reduced about 12% from mag. × 100.



Gross examination of the spleen disclosed enlargement and, generally, an increased firmness. In occasional spleens single or multiple infarcts were present. Microscopically, large amounts of homogeneous hyaline material were present throughout the splenic sinusoids. This material was similar in staining reactions to that described in the kidneys.

The lungs grossly showed no consistent changes, although hemorrhages were observed occasionally. Microscopically, small and large pulmonary arteries showed the presence of thrombi or emboli composed of hyaline material with varying admixtures of erythrocytes and leukocytes (Fig. 3). In an occasional animal, similar-appearing material was observed beneath the endothelium of the pulmonary arteries.

The hearts grossly showed no alterations. Microscopically, scattered areas of muscle necrosis and hemorrhage, with an associated cellular reaction of mononuclear and heterophilic cells, were observed frequently

Fig. 3.—Pulmonary artery which contains intraluminal thrombus or embolus in which there are numerous enmeshed erythrocytes and leukocytes. From a rabbit given 1200 r in air followed by endotoxin at 48 hours. Hematoxylin and eosin; reduced about 12% from mag. × 200.

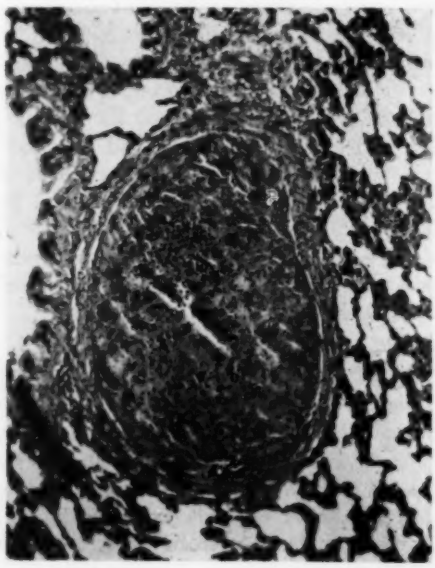


Fig. 4.—Section of the heart from a rabbit given 2400 r in air followed in 18 hours by endotoxin. There is extensive myocardial necrosis and inflammatory cellular reaction. Hematoxylin and eosin; reduced about 12% from mag. × 250.

Fig. 5.—Section from the mitral valve which shows extensive deposition of fibrinoid material in its substance; from a rabbit given 2400 r in air and endotoxin 18 hours later. Hematoxylin and eosin; reduced about 12% from mag. × 250.

Fig. 6.—Section of the liver from a rabbit given 1200 r in air followed by endotoxin 18 hours later. The section shows an area of extensive coagulative necrosis. Hematoxylin and eosin; reduced about 12% from mag. × 65.

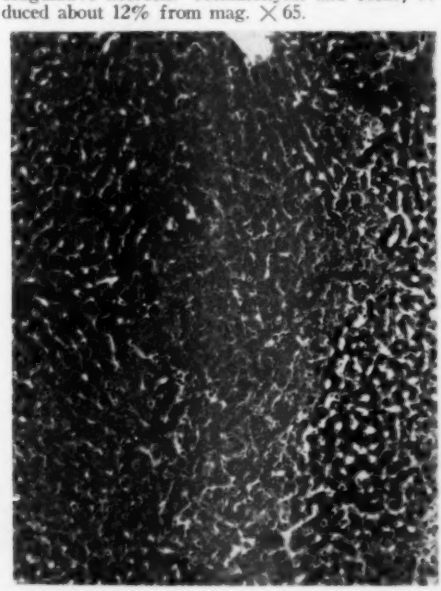
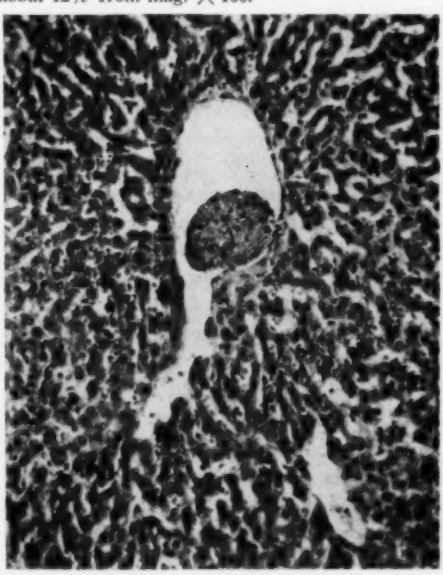


Fig. 7.—Section of the liver from another animal given 2400 r in air followed by endotoxin in 18 hours. The efferent vein shows the presence of a hyaline thrombus which appears to be covered by endothelium. Hematoxylin and eosin; reduced about 12% from mag. × 100.



(Fig. 4). Valvular hemorrhages were also observed occasionally. In conjunction with these changes, hyaline fibrinoid material was present beneath the endothelium of the intramural coronary arteries and in the substance of the valves (Fig. 5). In addition, many of the arteries showed the presence of intraluminal hyaline material which was associated with endothelial proliferation. In other arteries no fibrinoid material was observed, but the intima and media of the vessels appeared edematous and vacuolated.

No gross liver changes were noted, but areas of focal necrosis were seen frequently on microscopic examination. These areas of necrosis were surrounded by a cellular reaction which consisted predominantly of mononuclear cells. In many animals the areas of necrosis involved two or more contiguous lobules. In these areas the architectural pattern of the liver was preserved, but there appeared to be complete ischemic necrosis of all elements (Fig. 6). In most of the animals, deeply acidophilic hyaline thrombi were present in efferent veins. These thrombi were similar to the hyaline material described in the kidneys, spleen, lungs, and heart and stained in a similar fashion (Fig. 7).

No gross or microscopic changes were observed in the pancreas, adrenal gland, skeletal muscle, or intestinal tract.

Comment

Several previous papers have described in detail the morphologic changes in rabbits given Gram-negative endotoxin, alone or in conjunction with various other, apparently unrelated, substances. In each of these procedures diffuse vascular lesions, characterized by the presence of hyaline fibrinoid material, are produced in the kidneys, spleen, liver, lungs, and heart.[1-10]

In the present study it has been shown that entirely similar vascular lesions may be produced in rabbits given a single intravenous injection of endotoxin after exposure to abdominal radiation. Bilateral renal cortical necrosis, similar to that observed after two properly spaced injections of endotoxin, was observed also in the present experiments.

In rabbits in which these vascular lesions are produced by conventional means, such as by two injections of endotoxin or a single injection of endotoxin in association with the administration of sodium polyanetholsulfonate, the reaction is an uncontrolled one associated with massive deposits of fibrinoid material and the production of extensive areas of ischemic necrosis. Data obtained in the present studies suggest that the frequency, severity, and extent of such vascular lesions may be altered by modifying the amount of radiation or by varying the interval between irradiation and the administration of endotoxin.

By appropriate methods, the incidence of renal vascular lesions closely approximates that observed after two properly spaced injections of endotoxin. Reference to Table 5, for example, shows that of eight rabbits given endotoxin 18 hours after 2400 r in air, 87% developed renal fibrinoid lesions, 100% developed splenic lesions, and 50% developed vascular lesions of the heart, liver, and lungs. A comparable number developed fibrinoid lesions when given 1200 r in air followed in 24 hours by an intravenous injection of endotoxin. It appears from these studies, therefore, that certain combinations of radiation and endotoxin may provide a means of controlling the extent and severity of the vascular changes.

Previous studies have indicated that fibrinoid material is deposited in sites of vascular damage or endothelial alteration. The observation that fibrinoid was present in the walls of the renal interlobular arteries and afferent arterioles in many animals in this series, without tubular necrosis, suggests that irradiation may produce localized changes in the vascular system which allow this material to be deposited.

Except for the lateral bases of both lungs, which received direct radiation in some of the animals, the heart and lungs were ex-

cluded from direct radiation and received only scatter rays. It should be noted also that microscopic sections of the lungs were prepared from blocks from the upper lobes. The increased incidence of cardiac and pulmonary vascular lesions in animals given higher quantities of radiation is of interest. The fact that splenic fibrinoid lesions were observed in animals given large quantities of radiation alone is also of interest. These data suggest that, in addition to local vascular effects, large doses of abdominal radiation may have widespread effects and may be associated with systemic vascular alterations which provide foci for the deposition of fibrinoid.

The morphologic changes which are produced by combinations of abdominal irradiation and Gram-negative endotoxin are similar to those produced by two injections of toxin and suggest that the action of irradiation may be similar to that of an intravenous injection of endotoxin.

The mechanisms of action of endotoxin have not been clearly elucidated, although considerable information has been obtained in the past few years. It has been shown, for example, that a single intravenous injection of toxin is followed by the appearance of a cold heparin-precipitable protein fraction which appears to be an altered fibrinogen.[21,22] This material, as well as total plasma fibrinogen, precipitously declines when one of the high-molecular-weight polymers such as sodium polyanetholsulfonate is given, and the decrease in these plasma protein fractions has been correlated with the development of vascular fibrinoid lesions.[22] These data indicate that fibrinogen is involved in the production or formation of fibrinoid or its precursors. This theory is further supported by the observation that fibrinoid lesions are prevented by large amounts of heparin.[23] Preliminary studies indicate that abdominal irradiation does not produce the heparin-precipitable fraction but that there are alterations in total plasma fibrinogen and that the lesions can be prevented by giving large amounts of heparin.[24]

Another factor to be considered in the development of such radiation-toxin lesions is the possible occurrence of anticoagulants after radiation.[25] The pathogenesis of the hemorrhagic state which occurs after severe radiation injury has not been adequately defined. It is felt by some investigators that hemorrhage is due to a decrease in the number of circulating platelets,[26] and it has been shown that the amount of available heparin may be reduced after total-body radiation.[27] However, it is possible that radiation may be associated with the production of anticoagulants similar to the high-molecular-weight polymers which combine with fibrinogen or altered fibrinogen to produce vascular lesions.

It is known that fibrinoid lesions similar to those described here may be produced in rabbits infected with Group A streptococci or pneumococci and given a single intravenous injection of endotoxin.[6] Therefore, the possibility that bacteremia may play a role in the development of the radiation-toxin lesions merits consideration.

It has been shown by Warren and Whipple that bacteremia does not develop in dogs until after the fourth day of radiation.[28] Studies by Vincent, Veomett, and Riley[29] showed that bacteremia due to Gram-negative micro-organisms developed in rats on the third and fourth days after radiation. If these results are applicable to rabbits, it would then appear that the factor of bacteremia is not of great importance. Furthermore, it should be emphasized that, in these studies, vascular lesions were present within 36 hours after radiation and that when endotoxin was given prior to irradiation the incidence of vascular fibrinoid lesions was no greater than that reported in animals given only a single intravenous injection of endotoxin.[1] Finally, it should be stressed also that vascular fibrinoid lesions were observed after endotoxin and irradiation in doses as low as 300 r in air to the abdomen, and it is felt that higher dosages of radiation are necessary to produce a bacteremia.

Summary and Conclusions

Diffuse vascular fibrinoid lesions were produced in rabbits by exposure to abdominal radiation in conjunction with the intravenous administration of Gram-negative endotoxin. These lesions involved the kidneys, spleen, liver, lungs, and heart. The character and distribution of the lesions resembled that which occurs in the generalized Shwartzman phenomenon.

The frequency and distribution of the lesions varied with the quantity of radiation and the interval of time between radiation and administration of endotoxin. The data suggest that proper combinations of radiation and toxin may provide a method of producing controlled localized vascular fibrinoid lesions. The high incidence of diffuse lesions also suggests that, in addition to local effects, high quantities of abdominal irradiation may produce systemic vascular changes. Several possible mechanisms of action concerning the pathogenesis of the lesions are discussed.

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REFERENCES

1. Brunson, J. G.; Gamble, C. N., and Thomas, L.: Morphologic Changes in Rabbits Following the Intravenous Administration of Meningococcal Toxin: I. Effects Produced in Young and in Mature Animals by a Single Injection, Am. J. Path. 31:489-499, 1955.

2. Brunson, J. G.; Thomas, L., and Gamble, C. N.: Morphologic Changes in Rabbits Following the Intravenous Administration of Meningococcal Toxin: II. Two Appropriately Spaced Injections; the Role of Fibrinoid in the Generalized Shwartzman Reaction, Am. J. Path. 31:655-668, 1955.

3. Thomas, L., and Good, R. A.: Effect of Cortisone on the Shwartzman Reaction: Production of Lesions Resembling the Dermal and Generalized Shwartzman Reactions by a Single Injection of Bacterial Toxin in Cortisone-Treated Rabbits, J. Exper. Med. 95:409-428, 1952.

4. Smith, R. T.; Thomas, L., and Good, R. A.: Generalized Shwartzman Reaction: V. Intravenous Injection of Colloidal Iron or Carbon on Response of Rabbits to Meningococcal Toxin, Proc. Soc. Exper. Biol. & Med. 82:712-715, 1953.

5. Good, R. A., and Thomas, L.: Studies on the Generalized Shwartzman Reaction: II. The Production of Bilateral Cortical Necrosis of the Kidneys by a Single Injection of Bacterial Toxin in Rabbits Previously Treated with Thorotrast or Trypan Blue, J. Exper. Med. 96:625-641, 1952.

6. Thomas, L.; Denny, F. W., Jr., and Floyd, J.: Studies on the Generalized Shwartzman Reaction: III. Lesions of the Myocardium and Coronary Arteries Accompanying the Reaction in Rabbits Prepared by Infection with Group A Streptococci, J. Exper. Med. 97:751-766, 1953.

7. Brunson, J. G.; Davis, R. L., and Thomas, L.: Morphologic Changes in Rabbits Following the Intravenous Administration of Meningococcal Toxin: III. Effects Produced by Endotoxin in Association with Certain High Molecular Weight Acidic Polymers, Am. J. Path. 31:669-685, 1955.

8. Thomas, L.; Brunson, J. G., and Smith, R. T.: Studies on the Generalized Shwartzman Reaction: VI. Production of the Reaction by the Synergistic Action of Endotoxin with 3 Synthetic Acidic Polymers (Sodium Polyanethol Sulfonate, Dextran Sulfate, and Sodium Polyvinyl Alcohol Sulfonate), J. Exper. Med. 102:249-261, 1955.

9. Thomas, L., and Good, R. A.: Studies on the Generalized Shwartzman Reaction: I. General Observations Concerning the Phenomenon, J. Exper. Med. 96:605-624, 1952.

10. Kane, D. J.; Good, R. A., and Thomas, L.: Histologic Studies of the Renal Lesion in the Generalized Shwartzman Phenomenon, Abstract, Anat. Rec. 112:349, 1952.

11. Altshuler, C. H., and Angevine, D. M.: Histochemical Studies on the Pathogenesis of Fibrinoid, Am. J. Path. 25:1061-1077, 1949.

12. Gronvall, J. A., and Brunson, J. G.: The Generalized Shwartzman Phenomenon in Rats, A. M. A. Arch. Path. 62:324-334, 1956.

13. Gamble, C. N., and Brunson, J. G.: Experimental Fibrinoid Lesions in Rabbits: Production by Cross Transfusion and Isolated Renal Perfusion, A. M. A. Arch. Path. 60:583-592, 1955.

14. Brunson, J. G., and Davis, R. L.: Systemic Fibrinoid Disease: Similarity to Experimental Lesions in Rabbits, A. M. A. Arch. Path. 60:593-609, 1955.

15. Klemperer, P.; Pollack, A. D., and Baehr, G.: Diffuse Collagen Disease: Acute Disseminated Lupus Erythematosus and Diffuse Scleroderma, J. A. M. A. 119:331-332, 1942.

16. Klemperer, P.: Concept of Collagen Diseases, Am. J. Path. 26:505-519, 1950.

17. Zuelzer, W. W.; Palmer, H. D., and Newton, W. A.: Unusual Glomerulonephritis in Young Children, Probably Radiation Nephritis: Report of 3 Cases, Am. J. Path. 26:1019-1039, 1950.

18. Smith, W. G., and Williams, A. W.: Irradiation Nephritis, Lancet 2:175-177, 1955.

19. Hartman, F. W.; Balliger, A., and Doub, H. P.: Experimental Nephritis Produced by Irradiation, Am. J. M. Sc. 172:487-500, 1926.

20. Glasser, O.; Quimby, E. H.; Taylor, L. S., and Weatherwax, J. L.: Physical Foundations of Radiology, Ed. 2, New York, Paul B. Hoeber, Inc. (medical book department of Harper & Brothers) 1952, p. 519.

21. Thomas, L.; Smith, R. T., and Von Korff, R.: Cold-Precipitation by Heparin of a Protein in Rabbit and Human Plasma, Proc. Soc. Exper. Biol. & Med. 86:813-818, 1954.

22. Thomas, L.; Smith, R. T., and von Korff, R.: Studies on the Generalized Shwartzman Reaction: VII. The Role of Fibrinogen in the Deposition of Fibrinoid After Combined Injections of Endotoxin and Synthetic Acidic Polymer, J. Exper. Med. 102:263-278, 1955.

23. Good, R. A., and Thomas, L.: Studies on the Generalized Shwartzman Reaction: IV. Prevention of the Local and Generalized Shwartzman Reactions with Heparin, J. Exper. Med. 97:871-888, 1953.

24. Unpublished data.

25. Rosenthal, R. L.: The Relationships Among the Hematopoiesis, Blood Coagulation, Hemorrhage and Mortality in the Guinea Pig After Total Body X-Irradiation, Blood 10:510-527, 1955.

26. Jackson, D. P.; Cronkite, E. P.; LeRoy, G. V., and Halpern, B.: Further Studies on the Nature of the Hemorrhagic State in Radiation Injury, J. Lab. & Clin. Med. 39:449-461, 1952.

27. Hewett, J. E.; Hayes, T. L.; Gofman, J. W.; Jones, H. B., and Pierce, F. T.: Effects of Total Body Irradiation upon Lipoprotein Metabolism, Am. J. Physiol. 172:579-587, 1953.

28. Warren, S., and Whipple, G. W.: X-Ray Intoxication, Bacterial Invasion of the Blood Stream as Influenced by X-Ray Destruction of Mucosal Epithelium of the Small Intestine, J. Exper. Med. 38:713-723, 1923.

29. Vincent, J. G.; Veomett, R. C., and Riley, R. F.: The Relation of the Indigenous Flora of the Small Intestine of the Rat to Post-Irradiation Bacteremia, J. Bact. 69:38-44, 1955.

Carcinoma of the Liver with Clonorchiasis

Report of a Case Presenting as Hematuria and Metastasis to Renal Pelvis

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In the differential diagnosis of hematuria, the possibility of primary carcinoma of the kidney is always taken into account. Although metastatic tumors of the kidney are not rare, tending to occur frequently from primaries characterized by hematogenous spread, these are usually located in the cortex of the kidney and are not commonly associated with renal signs and symptoms.[1] The case to be presented is felt to be instructive on several counts. Firstly, the patient presented with hematuria and without other distracting complaints. Secondly, attention was drawn away from the possible presence of a tumor by the existence of a large staghorn calculus in the affected kidney. Finally, the metastatic tumor was only present within the renal pelvis, a very rare finding, and the primary hepatic carcinoma was associated with an infestation by the liver fluke Clonorchis sinensis.

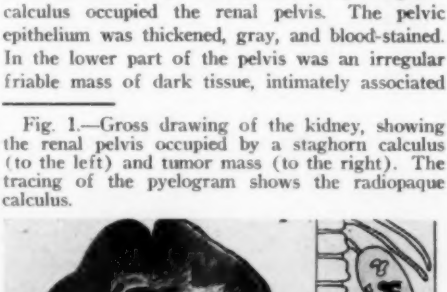
Report of Case

A 60-year-old Chinese man was admitted to the Urology Service of the Royal Victoria Hospital with complaints of hematuria, dysuria, and 18 lb. weight loss over the previous 10 months. He had been born in China but had lived in Canada since the age of 15 years. The history was otherwise noncontributory. Hematuria had been intermittent until two weeks before admission, at which time it became constant, with blood and clots in each urination. There had also been nocturia (three or four times a night) for the preceding eight months.

Physical examination revealed some tenderness in the left loin. The prostate was firm, of glandular consistency, and about double normal size. The liver was palpable 2 fingerbreadths below the right costal margin. No abdominal mass was felt. The hemoglobin was 14.5 gm/100 ml.; nonprotein nitrogen, 19 mg/100 ml. A sulfobromophthalein (Bromsulphalein) test indicated abnormal retention of the dye but was done postoperatively, and its significance is doubtful. The patient was not jaundiced. Cystoscopy revealed a normal lower urinary tract. Retrograde pyelography showed the presence of a staghorn calculus, occupying the lower half of the left kidney. The pyelogram of the right side was normal. A survey type chest x-ray, done on admission, was negative. Left nephrectomy was decided on and carried out without complication.

The kidney removed was externally unremarkable and of normal size and shape. When cut, it revealed a parenchyma which was hemorrhagic in places but other wise negative. The calyces were all moderately dilated, and a large staghorn calculus occupied the renal pelvis. The pelvic epithelium was thickened, gray, and blood-stained. In the lower part of the pelvis was an irregular friable mass of dark tissue, intimately associated

Fig. 1.—Gross drawing of the kidney, showing the renal pelvis occupied by a staghorn calculus (to the left) and tumor mass (to the right). The tracing of the pyelogram shows the radiopaque calculus.



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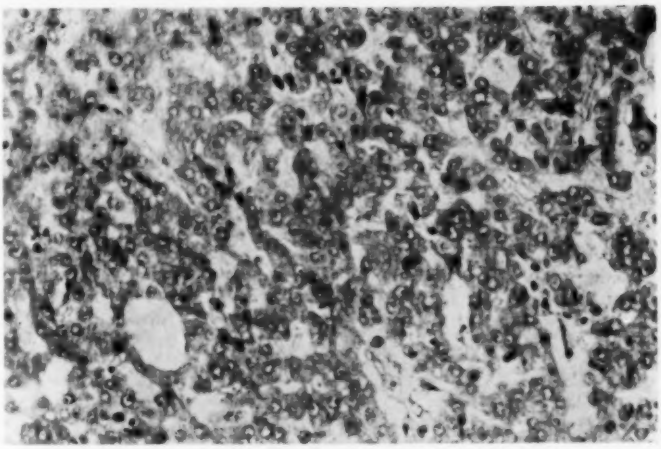

Fig. 2.—Structure of tumor mass in the renal pelvis, showing irregular plates and cords of cells with sinusoidal blood-filled spaces. Hematoxylin and eosin; reduced 40% from mag. × 660.

with the calculus. When this mass was cut, it was seen to have a marbled gray and red-black structure, with areas of necrosis. It measured roughly 5×2×2 cm., and, although loosely adherent to the pelvic epithelium in some areas, it could not be shown grossly to have any direct continuity with the actual renal parenchyma (Fig. 1). The attached portions of ureter and blood vessels were normal.

Microscopically, the kidney showed a chronic and acute pyelonephritis of nonspecific type. The pelvic epithelium was inflamed and eroded, but there was no squamous metaplasia. Sections of the intrapelvic mass bore a remarkable resemblance to well-differentiated liver tissue, complete with interlacing "plates" of epithelial cells and sinusoidal spaces lined by reticuloendothelial-like cells and even containing blood (Fig. 2). To complete the similarity, there were clear-cut little intercellular canaliculi containing plugs of golden-brown bile-like pigment (Fig. 3). This pigment was negative to an iron stain but gave a positive histochemical reaction for bilirubin, (by the Stein procedure).[2] No duct-like structures were seen. In some places this bile-forming tumor tissue replaced the pelvic epithelium, but nowhere could it be shown to invade the renal parenchyma. No tumor was found in the abundant subepithelial blood vessels or lymphatics.

It was concluded that this was a metastasis to the renal pelvis from a well-differentiated liver-cell carcinoma. The possibility of a choristoma

Fig. 3.—Detail of tumor cells, showing bile plug slightly above center. Hematoxylin and eosin; reduced 40% from mag. × 3600.

of liver tissue, arising in the kidney, was untenable on embryologic grounds.

Chemical analysis of the calculus revealed a composition of apatite (60%) and struvite (40%).

The patient made an uneventful recovery from his operation and was discharged on the 14th postoperative day.

Three months later he was readmitted to the medical service with right upper abdominal pain, anorexia, jaundice, and light-colored stools. At this time, he was found to be emaciated and severely jaundiced, with coarse rales throughout the right lung. The liver was palpable 10 cm. below the right costal margin. The hemoglobin was now 6 gm/100 ml., and the white blood cell count, 22,000 per cubic millimeter. He deteriorated rapidly and died in a few hours.

Autopsy Findings.—Autopsy was done 14 hours after death, and there was fairly severe autolysis of all tissues. The liver showed nodules of hepatic-cell carcinoma and a mild degree of portal fibrosis. It weighed 2475 gm. All biliary passages in and outside the liver contained flukes identified as C. sinensis. These organisms were also found in the duodenum and stomach. There were gallstones in the gallbladder and common duct. Metastatic tumor nodules were present in both lungs and adrenals, but no lymph nodes were involved. There was severe edema and congestion of the lungs. The remaining kidney was unremarkable except for a minute hemangioma. Other autopsy findings were not pertinent.

Comment

This man had been domiciled in Canada for 45 years, and it seems most likely that he had brought his liver flukes with him from his homeland. A more recent infestation via imported Chinese raw-fish delicacies is possible but less probable. The commonest precursor of primary liver-cell carcinoma, namely, portal fibrosis, was not a prominent feature in this case. No very clear relationship has ever been drawn between the high incidence of liver-cell carcinoma in some groups of Orientals and the prevalence of clonorchiasis. Berman's work in Africa suggests that diet may be a large factor.[3] Extensive work has been done on the carcinogenic effects of certain specific dietary factors, notably butter yellow,[4] and the diets of some Orientals apparently contain large amounts of such substances. Liver tumors have also been reported in man and animals in cases of infestation with the fluke Opisthorcus felineus,[5-7] which occurs in East Prussia, Russia, and Siberia. It is doubtful that the incidence of tumors in such infestations is more than coincidental, as with the schistosomes. At best it may be consequent to the irritative and fibrotic processes.[7]

It is unusual for a liver-cell carcinoma to metastasize to the kidney or adrenal, the common sites being lung and lymph nodes. None of Edmondson's[8] series had renal metastases and only six of Berman's.[3] The spread in this case was evidently hematogenous, as none of the body lymph nodes were involved. The lung nodules at autopsy were small, and the preoperative chest x-ray negative; thus tertiary spread from the lungs is unlikely.

Metastasis of any kind of tumor to the pelvis of the kidney is extremely rare. Hückel, in 1934, cited only two cases in the literature, both of which originated from the prostate.[9] In addition, he showed a photograph of a previously unpublished case of Lubarsch, in which an ovarian carcinoma had metastasized thus. Higgins' review of 47 tumors of the renal pelvis included no secondaries.[10] The ureter also is a rare site of metastases, although not so much so as the renal pelvis, Scott having listed 38 acceptable cases from the literature.[11]

In this particular case, it is interesting to speculate whether the presence of the staghorn calculus and consequent inflammatory changes in the renal pelvis might have made it a more fertile bed for the growth of tumor cells than would otherwise have been the case.

Summary

A case is presented of an elderly Chinese man with primary carcinoma of the liver and metastasis to the renal pelvis. The latter lesion was associated with a staghorn calculus, and these combined to produce the presenting complaint of hematuria. There were no signs or symptoms of liver disease until shortly before death. Autopsy revealed, in addition to the carcinoma, a heavy

infestation of the liver with Clonorchis sinensis.

Dr. Fred Stewart, Pathologist, Memorial Center for Cancer and Allied Diseases, New York, and Dr. Hugh Edmondson, Professor of Pathology, University of Southern California School of Medicine, Los Angeles, examined the original kidney slides.

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REFERENCES

1. Deming, C. L., in Urology, edited by M. Campbell, Philadelphia, W. B. Saunders Company, 1954, p. 1013.

2. Stein, J.: Réaction histochimique stable de détection de la bilirubine, Compt. rend. Soc. biol. 120:1136-1138, 1935.

3. Berman, C.: Primary Carcinoma of the Liver: A Study in Incidence, Clinical Manifestations, Pathology and Ætiology, London, H. K. Lewis & Co., Ltd., 1951.

4. Wilbur, D. L.; Wood, D. A., and Willett, F. M.: Primary Carcinoma of the Liver, Ann. Int. Med. 20:453-485, 1944.

5. Hoogland, H. J. M.: Carcinome der Gallenwege bei Distomatose der Katze, Ztschr. Krebsforsch. 29:239-269, 1929.

6. Ruditzky, M. G.: Zur Frage über die Entstehung des Leber- und Pankreaskrebses im Zusammenhang mit Distomatose, Ztschr. Krebsforsch. 27:402-416, 1928.

7. Stewart, M. J.: Precancerous Lesions of the Alimentary Tract, Lancet 2:699-675, 1931.

8. Edmondson, H. A., and Steiner, P. E.: Primary Carcinoma of the Liver: A Study of 100 Cases Among 48,900 Necropsies, Cancer 7:462-503, 1954.

9. Hückel, R., in Handbuch der speziellen pathogischen Anatomie und Histologie, edited by F. Henke and O. Lubarsch, Berlin, Julius Springer, 1934, Vol. 6, Pt. 2, p. 585.

10. Higgins, C. C.: Tumors of the Renal Pelvis: Review of 47 Cases, Ann. Surg. 137:195-204, 1953.

11. Scott, W. W., in Urology, edited by M. Campbell, Philadelphia, W. B. Saunders Company, 1954, p. 1040.

The Incidence and Etiology of Pulmonary Infarction in the Absence of Congestive Heart Failure

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Since Virchow noted the increase in size of the bronchial artery following pulmonary arterial occlusion,[1] investigators have been debating the functional importance of these blood vessels, especially their relative importance in the pathogenesis of pulmonary infarction. Because Virchow could not produce infarcts experimentally by pulmonary arterial occlusion alone, he concluded that the bronchial artery could maintain the integrity of the lungs in these cases. Other workers following Virchow agreed with this conclusion,[2-4] and it was generally accepted that a compromise of both pulmonary and bronchial circulations was necessary to produce an infarct. Pulmonary vascular congestion plus occlusion of the pulmonary artery, most often embolic, has been the commonest cause of such compromise. Since pulmonary vascular congestion is virtually always the result of left-sided heart failure, the concept was evolved that pulmonary infarcts in general did not occur in the absence of congestive heart failure. However, pulmonary infarcts in the absence of congestive heart failure have been noted periodically. Pleural effusion was stated to be a predisposing factor in some cases, while others were explained on the basis of "cachexia and debilitation." Some infarcts remained, the pathogenesis of which was not explained.

This study was undertaken to determine the actual frequency of pulmonary infarcts in the absence of congestive heart failure and to determine the reason for their occurrence.

Submitted for publication Dec. 23, 1957.

From the Laboratory Division, Montefiore Hospital. Trainee, National Cancer Institute.

Method of Study

A total of 2250 autopsies performed at Montefiore Hospital between 1950 and 1956 were reviewed. One hundred eighteen cases of complete embolic occlusion of a branch of the pulmonary artery were found and included in this study. Only embolic occlusions were included, since thrombi introduce factors of gradual occlusion and incomplete occlusion, both of which can alter the response of the pulmonary collateral circulation.

All pulmonary infarcts included in this series were examples of hemorrhagic infarction. Several lesions comparable to the incomplete infarct of Hampton and Castleman[5] were noted but were not included. The anatomic evidence of cardiac pathology at necropsy was considered indicative of cardiac disease regardless of clinical correlation. The determination of heart failure was somewhat more difficult. Heart failure was considered present if necropsy revealed acute or chronic passive congestion of the lungs or pulmonary edema in the presence of heart disease. However, since clinically controlled cardiac decompensation might show few or none of these anatomic findings, a review of the clinical record was indicated in some cases. Included in this study were a review of the medical history and physical examination on admission, venous pressure and circulation time (if performed), chest roentgenograms (with emphasis on the cardiac silhouette and evidence of pulmonary congestion), electrocardiogram, and, finally, a careful review of the clinical course in the hospital.

Results

The statistical analysis of the 118 cases is presented in the accompanying seven tables. As noted in Table 1, the age distribution corresponded with previous statistical studies.[6-7] Eighty-seven per cent of all cases occurred between the ages of 41 and 80 years, and forty-seven per cent, between 51 and 70 years of age. There was no sex prevalence (Table 2). Since pulmonary infarcts have been related to the function of the cardiopulmonary circuit, all cases have

TABLE 1.—*The Age Incidence of Pulmonary Emboli*

Age	No.
0–10	1
11–20	1
21–30	8
31–40	8
41–50	18
51–60	28
61–70	28
71–80	23
81–90	3
Total	118

been separated into those occurring in heart disease with failure, those occurring in heart disease without failure, and those occurring without associated heart disease (Table 3). The largest group comprised heart disease with congestive heart failure (45% of all cases). Of these, 90% had infarcts, and presumably infarcts were absent in the remainder only because there was insufficient time for their development prior to death. In 18% of the cases there was heart disease without congestive heart failure, and only 33% of these cases developed infarcts. This group included those cases in which the heart disease was an incidental or secondary finding at necropsy and not directly related to the patient's death. Functionally, these cases should be classified in the group of no heart disease, since they represent instances of only potential congestive heart failure. They are listed separately to avoid a possible source of error. The last group, comprising 36% of all the cases, had no heart disease, yet 47% of them had pulmonary infarcts. It was this group of pulmonary infarcts, occurring in patients with no demonstrable heart disease, that was of interest, since the predisposing factor of passive congestion of the lungs was absent. This group represented 17% of all cases and 27% of all infarcts.

TABLE 2.—*The Sex Incidence of Pulmonary Emboli*

	No.
Male	59
Female	59
Total	118

TABLE 3.—*The Distribution of Pulmonary Emboli and Infarction with Reference to Cardiopulmonary Function*

	Emboli, Total No.	Infarct, No.	Infarct, No.
Heart disease with failure	54	48	6
Heart disease without failure	21	7	14
No heart disease	43	20	23
Total	118	75	43

TABLE 4.—*The Age Incidence of Pulmonary Infarcts Occurring in the Absence of Heart Disease*

Age	No.
0–10	0
11–20	0
21–30	1
31–40	3
41–50	2
51–60	5
61–70	7
71–80	2
81–90	0
Total	20

Because of the particular interest in this group, the age and sex incidence were reevaluated as shown in Tables 4 and 5. These findings corresponded to those of the entire series of cases. In Table 6, the primary disease process in each of these cases has been listed. In one case a pulmonary

TABLE 5.—*The Sex Incidence of Pulmonary Infarcts Occurring in the Absence of Heart Disease*

	No.
Male	10
Female	10
Total	20

TABLE 6.—*Primary Disease Processes in Cases of Pulmonary Infarction Without Heart Disease*

Disease		No.
1. Carcinoma		16
(a) Lung	5	
(b) Stomach	4	
(c) Pancreas	3	
(d) Breast	2	
(e) Esophagus	1	
(f) Cervix	1	
2. Lymphoma (Hodgkin's disease)		1
3. Neurological disease		2
(a) Parkinsonism		
(b) Multiple sclerosis		
4. Pulmonary infarction		1
Total		20

TABLE 7.—*The Incidence of Predisposing Factors in Pulmonary Infarction Occurring in the Absence of Heart Disease*

No.	Pleural Effusion	Bronchial Obstruction	Atelectasis	Pneumonia	Multiple Emboli	Shock
12,137					×	×
12,151				Lipoid	×	×
12,157					×	×
12,296	×				×	
12,304			×		×	
12,441					×	×
12,553	×				×	×
12,609	×					×
12,710	×		×	×	×	×
12,738				×	×	
12,907	×				×	
13,317	×		×		×	
13,387	×		×			×
13,682					×	
13,691					×	×
13,731				×	×	
13,823				×	×	
13,932	×				×	
13,991		×			×	
14,143					×	×
Total	8	1	4	5	18	10

infarct secondary to thrombosis of the calf veins with embolism was the only pathologic sequence. Seventeen of the remaining cases had a malignant neoplasm, and ten of these involved the lung either as a primary or as a metastatic lesion. A predisposing relationship between a tumor and an infarct was considered to exist in only one instance. The remaining pulmonary tumors were either of insufficient size or of improper location to be a factor in the pathogenesis of the infarcts.

Other disease states which are considered to be a factor in the pathogenesis of pulmonary infarction are listed in Table 7. It is noteworthy that in 19 of the 20 cases occurring without associated heart disease two or more of these conditions were present. In the remaining case the infarct occurred in a segment of lung in which numerous emboli had lodged. The inclusion of shock as a predisposing factor may arouse some discussion. The mechanism of such predisposition will be described later. Terminal shock was never regarded as a predisposing factor, since there would be insufficient time for an infarct to develop. Therefore, episodes of shock were considered a predisposing factor only when they occurred at a time interval before death which was thought compatible with the age of the infarct at necropsy. Fifty per cent of the cases unassociated with heart disease had a documented episode of this type.

Comment

The results show that pulmonary infarction occurs in the absence of congestive heart failure. Actually, Hampton and Castleman [5] stated that 30% of 370 autopsies with pulmonary embolism or infarction occurred in noncardiacs, of whom 62% had had infarcts. The problem then exists as to how sufficient compromise of the circulation of the lung developed, in the absence of heart failure, to result in pulmonary infarction following embolization. Experimental production of pulmonary infarction has been attempted for more than 100 years. Many of these experimental data have been contradictory and, as a consequence, extremely confusing. One fact, upon which all agree, is that the pulmonary circulation is extremely complex and, in an otherwise normal lung, simple occlusion of a pulmonary artery will not lead to pulmonary infarction. Therefore, a basic understanding of the pulmonary circulation is essential.

It is well known that the arterial circulation is furnished by both the bronchial and the pulmonary arteries. It is also accepted that, apart from an insignificant amount drained by the bronchial veins, this blood is returned to the left side of the heart by

the pulmonary veins. Within the lung there are numerous anastomoses between the three major blood vessels, namely, the bronchial and pulmonary arteries and the pulmonary vein,[4,8,9] in addition to the capillary network. Liebow has also demonstrated bronchopulmonary venous shunts in emphysema.[17] Therefore, the basic anatomic pattern consists of a huge anastomotic network with two major sources of supply and one major source of drainage. Some investigators have disclaimed the role of the bronchial artery in the pathogenesis of pulmonary infarcts.[10,11] Since the bronchial artery can maintain the integrity of the lung after ligation of the main pulmonary artery,[3,4] it must be assumed it is also a factor in preventing infarction after occlusion of a smaller pulmonary artery as well. Because of its size this role may be less important than that of the pulmonary artery and vein, but in a marginal state the bronchial artery may be extremely important in affecting the viability of a segment of lung. Clinically as well as experimentally, if two or more of these major vascular channels are compromised, infarction will often result, provided sufficient time elapses.[10-13] The only reported instance in which infarction followed obstruction of one major channel was a case reported by Pecora.[14] In this case infarction of the lung followed surgical ligation of the main pulmonary artery. Because of the diffuse anastomotic network, the situation is somewhat different when a segment of pulmonary parenchyma smaller than a lobe is involved. There must be a compromise of the anastomotic circulation in addition to occlusion of the main artery supplying such a segment. Passive congestion of the lungs, because of its diffuse nature, achieves such a result. If other conditions are to predispose to infarction, they too must involve a considerable portion of pulmonary parenchyma adjacent to the involved segment of lung. Each of the factors listed in Table 7 exerts such an influence. These factors can be arbitrarily classified as extrapulmonary, parenchymal, and circulatory.

1. *Extrapulmonary Factors.*—The first factor, pleural effusion, exerts hydrostatic pressure on the lung. This force initially results in atelectasis, but as the pleural effusion increases it results in vascular collapse as well. Karsner [10] has experimentally produced infarcts following artificial effusion and pulmonary arterial occlusion. Clinically, pleural effusion would be a predisposing factor chiefly in the lower lobes except in instances of massive effusion. This is extremely important, since most emboli lodge in the lower lobes. A diagnostic problem exists at necropsy, since, in addition to being a predisposing factor to infarction, pleural effusion may result from an infarct. Differentiation was established on the character and volume of the pleural effusion in these cases.

2. *Parenchymal Factors.*—Pulmonary parenchyma is unique, since by its nature it can exchange gaseous metabolites directly with the atmosphere and indirectly by means of the blood stream. After bronchial obstruction, however, the pulmonary circulation remains as the only medium of such exchange. Under these circumstances any compromise of the circulation would be more critical than in a normal state. Bruner [13] has postulated that this situation predisposes to infarction, and Karsner [10] has demonstrated this experimentally. In this series there is one instance of such a circumstance, which resulted from a large hilar tumor obstructing the bronchi. Atelectasis may predispose to infarction only by nature of its etiology, which may be bronchial obstruction or an external force, such as pleural effusion.

The circulatory changes in pneumonia, as demonstrated by Mathes et al.,[15] consist chiefly of thrombosed capillary channels. Such a condition, if extensive, would effectively reduce the collateral circulation and thereby predispose to infarction. Roach and Laufman [16] concluded that distemper, an analogous condition in dogs, does predispose to the experimental production of pulmonary infarcts. The circulatory changes

in emphysema and bronchiectasis have been studied by Liebow et al.[17,18] In emphysema bronchopulmonary venous shunts are described which should not predispose to infarction. However, in emphysema numerous alveolar septa are ruptured which reduce the size of the capillary bed and the collateral circuit. This may have an effect in favoring the development of an infarct. Since pulmonary fibrosis usually connotes destruction of pulmonary parenchyma, a similar effect may also be present in this condition. In bronchiectasis bronchopulmonary arterial shunts are formed which should help prevent the development of infarcts. In this series emphysema, pulmonary fibrosis, and bronchiectasis were not significant factors in the production of pulmonary infarcts. The circulation of neoplasm of the lung has been studied by Wright[19] and found to be derived from the bronchial artery. In the absence of bronchial or vascular compression, however, the role of pulmonary tumor in the pathogenesis of infarction is obscure.

3. *Circulatory Factors.*—Ellis et al.[11] have demonstrated the experimental production of infarcts as a result of multiple emboli. Certainly the effectiveness of an anastomotic network in a given segment of lung would be markedly impaired if the adjacent vessels supplying this network were also occluded. In this series there was one instance in which numerous emboli were present without other predisposing factors. Associated with many cases of pulmonary embolization is an episode of shock and, more specifically, a drop in blood pressure. According to Parin,[20] this is secondary to peripheral arteriolar dilatation which follows a sudden rise in pressure of the pulmonary artery proximal to the occlusion. This peripheral arteriolar dilatation involves the bronchial artery as well. If the pressure in the bronchial artery falls, the collateral circulation of the involved segment of lung from this source proportionately diminishes.[21] If one accepts the bronchial circulation as a factor in preventing pulmonary infarction, the effect of shock in minimizing the effectiveness of this factor must also be accepted. Therefore, in the absence of other parenchymal or extrapulmonary factors, it appears possible to develop pulmonary infarcts solely from multiple pulmonary emboli and shock. Indeed, six of the cases of unexplained infarction in the present series fit into this category. Conclusive experimental evidence for this premise unfortunately is lacking. This concept would explain those cases of pulmonary infarcts occurring in otherwise normal lungs which hitherto were ascribed to such entities as cachexia and debilitation.

Summary and Conclusions

Pulmonary infarction can occur in the absence of congestive heart failure and in the absence of heart disease without decompensation; 27% of all infarcts in this study occurred in this group.

Congestive heart failure is the most frequent predisposing factor in the development of pulmonary infarction.

In the absence of congestive heart failure, pleural effusion, pneumonia, atelectasis, and bronchial occlusion can predispose to infarction.

It is proposed that multiple emboli and the associated shock cause sufficient impairment of the bronchial and pulmonary circulation in an otherwise normal lung to result in pulmonary infarction.

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REFERENCES

1. Virchow, R.: Gesammelte Abhandlungen zur wissenschaftlichen Medicin, Frankfurt, Meidlinger Sohn & Co., 1856, p. 219.

2. Kuttner: Beitrag zur Kenntniss der Kreislaufsverhältnisse der Säugethierlunge, Arch. Path. Anat. 73:476, 1878.

3. Schlaepfer, K.: Ligation of the Pulmonary Artery of One Lung With and Without Resection of the Phrenic Nerve, Arch. Surg. 9:25, 1924.

4. Liebow, A. A.; Hales, M. R.; Bloomer, W. E.; Harrison, W., and Lindskog, G. E.: Studies on the Lung After Ligation of the Pul-

monary Artery: Anatomical Changes, Am. J. Path. 26:177, 1950.

5. Hampton, A. O., and Castleman, B.: Correlation of Postmortem Chest Teleoroentgenograms with Autopsy Findings, with Special Reference to Pulmonary Embolism and Infarction, Am. J. Roentgenol. 43:305, 1940.

6. Miller, R., and Berry, J. B.: Pulmonary Infarction: A Frequently Missed Diagnosis, Am. J. M. Sc. 222:197, 1951.

7. Barnes, A. R.: Pulmonary Embolism, J. A. M. A. 109:1347, 1937.

8. Tobin, C. E.: The Bronchial Arteries and Their Connections with Other Vessels in the Human Lung, Surg. Gynec. & Obst. 95:741, 1952.

9. Ghoreyeb, A. A., and Karsner, H. T.: A Study of the Relation of Pulmonary and Bronchial Circulation, J. Exper. Med. 18:500, 1913.

10. Karsner, H. T., and Ash, J. E.: Studies in Infarction: II. Experimental Bland Infarction of the Lung, J. M. Res. 27:205, 1913.

11. Ellis, F. H.; Grindlay, J. H., and Edwards, J. E.: The Bronchial Arteries: II. Their Role in Pulmonary Embolism and Infarction, Surgery 31:167, 1952.

12. Steinberg, B., and Mundy, C. S.: Experimental Pulmonary Embolism and Infarction, Arch. Path. 22:529, 1936.

13. Bruner, H. D.: The Bronchial Artery in Pulmonary Infarction, North Carolina M. J. 8:306, 1947.

14. Pecora, D. V.; Brook, R., and Cooper, P.: Pulmonary Infarction Following Interruption of the Pulmonary Artery: Report of Case, J. Thoracic Surg. 29:572, 1955.

15. Mathes, M. E.; Holman, E., and Reichert, R. L.: A Study of the Bronchial, Pulmonary and Lymphatic Circulations of the Lung Under Various Pathologic Conditions Experimentally Produced, J. Thoracic Surg. 1:339, 1932.

16. Roach, H. D., and Laufman, H.: Relationship Between Pulmonary Embolism and Pulmonary Infarction: An Experimental Study, Ann. Surg. 142:82, 1955.

17. Liebow, A. A.: The Bronchopulmonary Venous Collateral Circulation with Special Reference to Emphysema, Am. J. Path. 29:251, 1953.

18. Liebow, A. A.; Hales, M. R., and Lindskog, G. E.: Enlargement of the Bronchial Arteries, and Their Anastomoses with the Pulmonary Arteries in Bronchiectasis, Am. J. Path. 25:211, 1949.

19. Wright, R. D.: The Blood Supply of Abnormal Tissues in the Lungs, J. Path. & Bact. 47:489, 1938.

20. Parin, V. V.: The Role of Pulmonary Vessels in the Reflex Control of the Blood Circulation, Am. J. M. Sc. 214:167, 1947.

21. Karsner, H. T., and Ghoreyeb, A. A.: Studies in Infarction: The Circulation in Experimental Pulmonary Embolism, J. Exper. Med. 18: 507, 1913.

Obituaries

GEORGE GOMORI, M.D.
1904-1957

Dr. George Gomori, born in Budapest, Hungary, July 16, 1904, died Feb. 28, 1957, of a myocardial infarction. This marked the untimely end of a brilliant and warm human being. He received his M.D. degree from Budapest in 1928 and his Ph.D. in Medicine from The University of Chicago in 1943. In Budapest he rose from junior assistant in surgery to assistant professor. In 1938 he came to this country and after a brief stay in a private hospital laboratory became assistant in medicine at The University of Chicago and rose from there to become Professor of Medicine in 1949. In 1956 he left The University of Chicago and moved to Palo Alto, Calif. He was a Fellow of the American Medical Association and a member of the Society for Experimental Biology and Medicine, the American Society of Clinical Pathologists, and the Central Society for Clinical Investigation. He was a founder and at the time of his death President of the Histochemical Society. He was on the editorial board of the *American Journal of Clinical Pathology* and the *Journal of Histochemistry and Cytochemistry*. In 1955 he received the Ward Burdick Award of the American Society of Clinical Pathologists recognition of his contribution to the field of pathology.

Several thoughtful and laudatory eulogies have already been written marking George Gomori's passing. In these several places his fine qualities as a human

GEORGE GOMORI, M.D.
1904-1957

being and his intellectual skills have been recounted. It seems, therefore, appropriate here to attempt to assess another important aspect of George Gomori's life; namely, the impact of his work in the present and its meaning to the future of biology and in particular pathology. In the introduction to his book "Microscopic Histochemistry" published in 1952, Dr. Gomori says, "Histochemistry is a borderline field between histology and analytical chemistry or biochemistry." He indicates further that histochemistry, and in particular that variety, microscopic histochemistry, which was his particular area was almost nonexistent until the late 1930's. Until then only a few chemical reactions, such as those for iron, starch, and fat, could be called truly histochemical. The majority of methods used to demonstrate cellular elements consisted of staining procedures concerning which little in the realm of chemistry was known. The name of Gomori along with that of Lison stands out in this beginning era, and the name of Gomori has continued to stand out through the intervening years. His first paper on alkaline phosphatase, published in 1939, marked the real beginning in enzymatic histochemistry. Here for the first time someone took an enzymatic activity described previously in test-tube reactions and attempted to define conditions under which a specific precipitate could be visualized at the site of its formation in a tissue section. In achieving this Gomori set the pattern of future development in this field. It is of interest to consider the fact that the information necessary to produce this demonstration had been present for a number of years before. It took insight and imagination to put the pieces together. Following along this path George Gomori and others have since been able to demonstrate not only alkaline and acid phosphatases but lipases and a variety of esterases, including acetylcholine esterase. In the case of the lipases Dr. Gomori had to search for several years among various fatty acid esters until he found a series among the commercial detergents which suited his conditions. In the case of certain of the esters of phenolic substances he again sought and synthesized fairly systematically a great many until he found those suited to the histochemical conditions. His usual approach to a problem was the following: Having selected a particular chemical linkage or chemical grouping which might be acted upon enzymatically, he would search for the compound which might work in the histochemical system. Sometimes he had stated reasons and sometimes only his intuition in the initial search. Having once established the workability of a particular chemical reaction, he would then set about systematically to modify the substrate. With any given substrate he always tried multiple tissues from a single animal species and also tried the tissues of different animal species. For this purpose he always kept a stock of "compound" blocks and cut tissues. On one slide there might be as many as seventeen to twenty organs and tissues neatly and prettily composited. He was always interested in the application of the new histochemical reactions developed in his laboratory to medical problems and regularly collected fresh material from the surgical pathology laboratory and autopsy material from the morgue. Frequently these were examined with the new method along with the animal tissues.

In addition to his studies on enzymes he examined the characteristics of other chemical reactions in microscopic sections. He confirmed and extended the notion developed by Lison and by Erspamer that the histochemical reactions characteristic of enterochromaffin cells were those of a phenolic substance. His original notion that it closely resembled resorcinol he was later willing to modify, and he conceded that it was 5-hydroxytryptamine, as Erspamer had suggested. Another reaction

which he examined was that of hydrazine derivatives in adrenal cortex, originally assumed to be due to the presence of ketosteroids. Here he was able to show clearly that the reaction was most likely due to the spontaneous oxidation of lipid material present in the adrenal cortex. These things bring out another of his characteristics, namely, that he was always ready to be convinced by suitable evidence. He was very critical of his own work and critical of the work of others. He was capable of lauding a good piece of investigation and of sharply criticizing that which he considered to be sloppy. He could simultaneously admire inventiveness and criticize sloppiness of detail.

In addition to his exploration of enzymatic microscopic histochemical reactions he explored a variety of enzyme reactions and nonenzymatic chemical reactions in the test tube. He also explored and invented new kinds of staining procedures which were not strictly histochemical; his trichrome stains and his chrome hematoxylin stain are widely used in histology and pathology. In his book almost every histochemical reaction about which he speaks was tried by his own hands and assessed as to its merit. In writing about his researches he was terse and though usually clear somewhat impatient about recording detail. In addition, he had the capacity shared by other bright minds of forgetting or not recognizing certain details which he performed instinctively or by force of habit. Some of these omissions have caused others less cognizant of the facts and less competent in their management to be critical of some of his methods.

George Gomori had the imaginative and the inventive mind combined with the scientific critical judgment, the ingredients of fruitful investigation. Although he applied histochemical techniques to many organs and tissues and to many species and pathologic processes, he did not do so blindly or wantonly. He did it always in an intelligently exploratory fashion, and what he considered inconsequential he did not publish. Although he considered that histochemistry had become firmly established as a science in its own right, nevertheless it was and is still a young science. He firmly believed that it would be the exploration of new methods, of new substrates, of new enzyme systems, and of new chemical reactions which would produce the fruit of this science in biology and in pathology. This was George Gomori's work, and this is the heritage which he leaves to those engaged in histochemistry to carry on.

EARL P. BENDITT, M.D.

News and Comment

PERSONAL

Award for Dr. James B. McNaught.—Dr. James B. McNaught, of Denver, was recently awarded a certificate of "highest merit" and a gold medallion at the annual meeting of the American Society of Clinical Pathologists. The award was made in recognition of his great contributions to clinical pathology.

Fourth Annual Ludvig Hektoen Memorial Lecture.—The 4th Annual Ludvig Hektoen Memorial Lecture was given by Dr. Cecil Watson, Chairman of the Department of Medicine of the University of Minnesota, at the Cook County Hospital, in Chicago, on Feb. 6, 1958. The title of the lecture was "Some Recent Studies of the Porphyria Problem."

ANNOUNCEMENTS

Conference on Chemical Organization of Cells.—A Conference on the Chemical Organization of Cells, Normal and Abnormal, will be held in Madison, Wis., on Aug. 21, 22, and 23, 1958.

Participants will be authorities on anatomy, biochemistry, embryology, and pathology, who will present correlated recent information about the cell in a series of formal presentations.

Inquiries should be addressed to Dr. Joseph J. Lalich, Professor of Pathology, University of Wisconsin, Madison 6, Wis.

A limited number of travel stipends may become available later this spring. Information will be advertised in appropriate journals and circularized to those who have already registered with Dr. Lalich for the conference.

Bertha Goldblatt Teplitz Award.—The Ann Langer Cancer Research Foundation announces an annual award of $500 for meritorious investigation in the field of cancer research, either clinical or laboratory. The award is being supported by the family of the late Bertha Goldblatt Teplitz and carries her name.

Competition shall be limited to physicians and other scientists, clinical or laboratory, under the age of 45. The name of the recipient of the 1958 award will be announced May 1, 1958.

The award committee consists of Dr. Austin M. Brues, Director of Biological and Medical Research, Argonne National Laboratory, and Professor, University of Chicago; Dr. Israel Davidsohn, Director of Laboratories and Research, Mount Sinai Hospital, and Professor, Chicago Medical School; Dr. Danely P. Slaughter, Director, Tumor Clinic, University of Illinois, and Professor, University of Illinois College of Medicine; Dr. Albert Tannenbaum, Director, Department of Cancer Research, Medical Research Institute, Michael Reese Hospital, and Dr. William B. Wartman, Chairman, Department of Pathology, Northwestern University Medical School.

Nominations should be submitted to Teplitz Award Committee, 612 N. Michigan Ave., Chicago 11, by March 15, 1958, and should be accompanied by a short one-page statement and biography.

Fellowships for Tissue-Culture Course.—The National Foundation for Infantile Paralysis is again offering fellowships to postdoctoral investigators, teachers, graduate students, and experienced laboratory personnel with the baccalaureate degree for participation in short courses in tissue culture.

Fellowships may be used for study only in formal courses designed to teach the principles, techniques, and application of tissue culture. Funds will be awarded for the period necessary to complete the course, which, in most instances, is not expected to exceed six weeks.

Financial assistance, based on individual need, may include an allowance for maintenance, for tuition and fees, and for round-trip transportation.

Further information and application forms may be obtained from the Division of Professional Education. Completed application should reach the National Foundation at least six weeks prior to the beginning of the course.

Write to Division of Professional Education, National Foundation for Infantile Paralysis, 301 E. 42d St., New York 17.

Supplement to "Survey of Compounds Which Have Been Tested for Carcinogenic Activity."—Supplement I to "Survey of Compounds Which Have Been Tested for Carcinogenic Activity" is now available. The publication lists 981 compounds which were tested during the period 1948 through 1953. Data were collected and classified by Prof. Philippe Shubik, of the Chicago Medical School, and Dr. Jonathan Hartwell, of the National Cancer Institute. Of the total number tested, 779 are reported for the first time in these tests. Copies of the new publication are available from the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. at $3.50 each.

Books

BOOK REVIEWS

Früherkennung des Collumcarcinoms: Leistungen und Grenzen der Kolposkopie, Cytologie und Histologie. Deutsche Gesellschaft für Gynäkologie. C. Kaufmann, conductor of symposium. Edited by H. Runge, K. G. Ober, and P. Stoll. Price, DM 12.80. Pp. 75, with 19 illustrations. Springer-Verlag, Reichpietschufer 20, (1) Berlin W. 35, 1957.

A symposium on the efficiency and limitation of cytology, colposcopy, and histology in the early detection of cervical carcinoma was held at the thirty-first meeting of the German Society of Gynecology in 1956. The papers given there and the discussions are now published under the auspices of C. Kaufmann, Cologne. The panel consisted of the most outstanding men in the field. In Germany, the country where colposcopy was introduced, cytology is regarded more effective than colposcopy in the detection of cervical carcinoma. The papers presented in this fascicle reflect rather controversial viewpoints of individual panel members. It is unfortunate that the papers were hardly discussed, probably owing to the limited time. It seems to this reviewer that it would have been better to limit the wide-range program and allow a thorough discussion of the disputable points.

Lehrbuch der allgemeinen Pathologie und der pathologischen Anatomie. By Herwig Hamperl. Price, DM 58. Pp. 773, with 686 illustrations. Springer-Verlag, Reichpietschufer 20, (1) Berlin W 35, 1954.

The great demand of German medical students had necessitated the 20th and 21st editions of Ribbert's popular textbook of general pathology and pathologic anatomy shortly after its previous edition. The book has for years been published and renewed by Dr. Herwig Hamperl, Professor of Pathology at the University of Bonn. The name of the author, well known in this country, is a guarantee of quality of book. "A suitable textbook of pathology for medical students should omit many things out of the multiplicity of facts and opinions" and "every author will proceed according to his own principles and aims." Therefore, well-established and subjectively selected fundamentals of general and organ pathology are treated precisely and in a lively German, while "the often changing interpretations and opinions" are left out. This is his privilege, and we are not to criticize him for certain shortcomings. However, an American reader will be annoyed by several flaws. For example, the chapter on fungus disease is very sketchy and incorrect; histological classifications of tumors (lung, breast) are antiquated. Why does he spend three pages on the intestinal manifestations of typhoid fever, while ulcerative colitis is scarcely treated? On the other hand, the introduction on the general aspects of tumors, leukemias, resistance, and many parts of the organ pathology are superbly done, though the text is often compressed. Noteworthy is the author's ability to develop clearly and logically pathogenetic principles in the treatment of inflammation or vascular renal diseases. The illustrations (black-and-white photographs, drawings) are of good quality and instructive. Magnifications of photomicrographs are mostly lacking. Certainly a great help to the beginner are the many translations of foreign terms and explanations of authors' names. The newly added references are mostly those of the German literature. The book is accurately printed on good paper.

Gout. Second edition. By John H. Talbot, M.D. Price, $6.75. Pp. 205, with 74 illustrations. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1957.

This little monograph has been colorfully written and magnificently illustrated. Considerations of the hereditary nature of this disease, its clinical picture, the intermediate metabolism of uric acid, renal exchanges of urates, the pharmacology of antigout drugs, and the treatment of gout have resulted in a monograph that deals with all aspects of gout. A more complete coverage of gout is probably not available in recent English medical literature.

Principles of Urology: An Introductory Textbook to the Diseases of the Urogenital Tract. By Meridith F. Campbell. Price, $9.50. Pp. 622, with 319 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1957.

The broad fundamentals of urology are presented in a very didactic, pedagogic, yet refreshingly clear manner. Basic anatomy and physiology are succinctly reviewed; diagnostic studies up to the point of major urosurgical intervention are carefully discussed, and minor urosurgical office procedures are outlined. Although this book was written primarily for the medical student and general practitioner, almost every physician would find numerous occasions to refer to it.

Clinical Pathology in General Practice. By the British Medical Association. Price, $5. Pp. 321, with 51 illustrations. J. P. Lippincott Company, 227-231 S. 6th St., Philadelphia 5, 1957.

This book was written for the purpose of acquainting the general practitioner with the most effective way of utilizing laboratory facilities. The interpretations of laboratory tests which are used to evaluate body function are discussed, and practical techniques for the handling of specimens are carefully outlined. Numerous pearls of information and that distinctively clear style of writing peculiar to so many British authors have been combined to make a book that would be useful to general practitioners and hospital pathologists.

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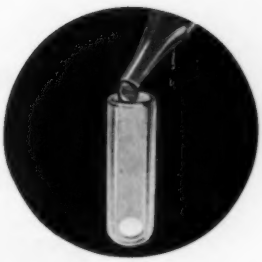
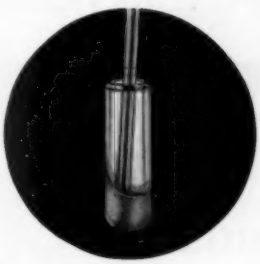
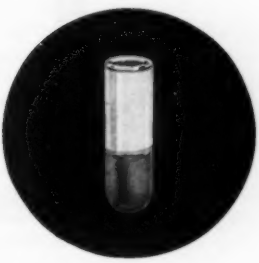


1. Add 4 drops serum or plasma to one PHOSPHATAB tablet in special tube.

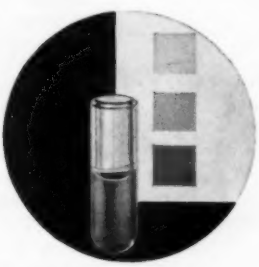


2. Crush tablet.



3. Let stand at room temperature 12-30 minutes. (time from chart)

4. Add 1 drop of color developer: mix.



5. Compare color.

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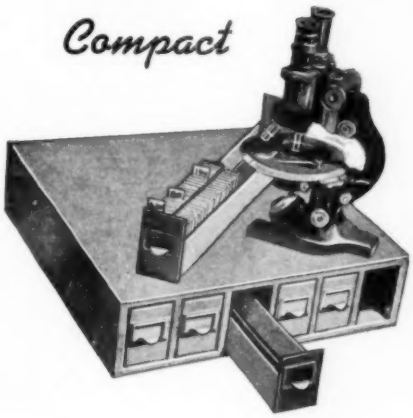

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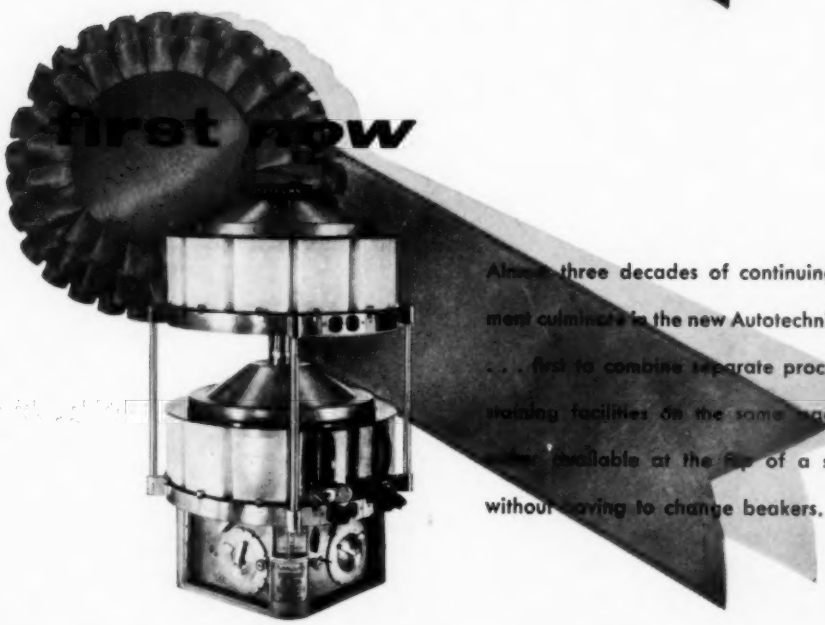

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